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GENETIC AND COGNITIVE ASPECTS ON RECOVERY AFTER PROPOFOL ANAESTHESIA

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Cover picture: The propofol molecule by Olivia Lindqvist

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Till mina små galningar Olivia, Klara och Simon

"Om snöret inte håller, utan går av, är det bara att försöka med ett annat snöre."

Nalle Puh (A.A Milne)

ABSTRACT

Propofol is one of the most used intravenous anaesthetics in the western world. It is often used for ambulatory surgery due to favourable pharmacokinetic properties allowing quick onset and short emergence time. However, there is considerable interindividual variation in pharmacokinetics and dynamics as well as gender differences. Differences in metabolism due to polymorphic enzymes may be a contributing factor to this variation. To enable early and smooth discharge from hospital after ambulatory surgery, a quick postoperative cognitive recovery is essential. It is not known whether the great variation in propofol pharmacokinetics and pharmacodynamics affect the cognitive recovery of the ambulatory patient receiving propofol.

By studying correlation between genotype and propofol metabolite production both in liver microsomes and in humans after propofol anaesthesia, we aimed to further describe the variations in propofol pharmacokinetics. Postoperative cognitive recovery in women undergoing ambulatory breast cancer surgery with propofol or desflurane anaesthesia was studied, using the PQRS and CFQ as assessment tools. Further the cognitive performance according to PQRS in a test re-test situation in pre-surgery cancer patients compared to controls was evaluated.

Our results demonstrate a great variation in production of propofol metabolites in vitro and in vivo, but no correlation between metabolite level and genotype. Females showed a higher propofol metabolite level compared to men after both bolus dose and infusion of propofol. Cognitive recovery was similar after propofol and desflurane anaesthesia, and subjectively not complete one week after surgery. We found that pre-surgery cancer patients expressed a higher level of anxiety and had lower cognitive baseline test performance compared to controls, resulting in a high exclusion rate in the patient group. The groups had a similar re-test performance in the PQRS cognitive domain.

In conclusion, we found a considerable variability in production of propofol metabolites but no correlation to genotype. There was an increased production of propofol metabolites in women compared to men. The protracted postoperative cognitive recovery assessed by PQRS and CFQ after ambulatory surgery was similar after propofol and desflurane anaesthesia, suggesting that possible remains of propofol or its metabolites do not affect cognitive performance more than residual effects of desflurane. When assessing postoperative cognitive recovery it should be acknowledged that the anxiety and stress caused by a severe disease and wait for surgery may have an impact on cognitive PQRS test performance. The use of the revised PQRS cognitive scoring system may lead to the exclusion of a considerable part of the patients due to too low baseline performance.

Key words; propofol, CYP2B6, UGT1A9, propofol metabolites, gender difference, postoperative cognitive recovery, desflurane, Postoperative Quality of Recovery Scale, Cognitive Failure Questionnaire

LIST OF PUBLICATIONS

This thesis has been based on the following papers, which will be referred to in the text by their roman numerals.

I. **Influence of sex on propofol metabolism, a pilot study: implications for propofol anesthesia**

   Loryan I*, Lindqvist M*, Johansson I, Hiratsuka M, van der Heiden I, van Schaik RH, Jakobsson J, Ingelman-Sundberg M

   Eur J Clin Pharmacol (2012) 68:397–406

II. **Sex difference in formation of propofol metabolites: a replication study**

   Choong E, Loryan I, Lindqvist M, Nordling A, El Bouazzaoui S, van Schaik RH, Johansson I, Jakobsson J, Ingelman-Sundberg M

   Basic & Clinical Pharmacology & Toxicology, 2013, 113, 126–131

III. **Cognitive recovery after ambulatory anaesthesia based on desflurane or propofol: a prospective randomised study**

   Lindqvist M, Schening A*, Granstrom A*, Bjorne H, Jakobsson JG

   Acta Anaesthesiol Scand 2014 Oct; (9): 1111-20

IV. **Cognitive baseline test and re-test performance according to the revised Postoperative Quality of Recovery Scale in pre-surgery cancer patients -a controlled study**

   Lindqvist M, Granstrom A*, Schening A*, Bjorne H, Jakobsson JG

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   * These authors contributed equally to the paper

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List of abbreviations

1-QG	Quinol-1-Glucuronide
4-QG	Quinol-4-Glucuronide
4-QS	Quinol-4-Sulphate
AAI	Auditory Evoked Potential Index
ASA	American Society of Anesthesiologists
AUC	Area Under the Curve
BIS	BISpectral monitoring
CFQ	Cognitive Failure Questionnaire
CNS	Central Nervous System
CYP	Cytochrome P
DNA	DeoxyRibonucleic Acid
FiO2	Fraction of Inspired Oxygen
GABA	G-Amino Butyric Acid
HLM	Human Liver Microsome
HPLC	High-Performance Liquid Chromatography
HRP	Horse Radish Peroxidase
IgG	Immunoglubulin G
IL-6	Inflammatory Interleukin 6
ISPOCD	International Study on PostOperative Cognitive Dysfunction
LOC	Loss Of Consciousness
LOVR	Loss Of Verbal Response
NADPH	Nicotinamide Adenine Dinucleotide PHosphate-oxidase
NMDA	N-Methyl-D-aspartate
PADSS	Post-Anaesthetic Discharge Scoring System
PCR	Polymerase Chain Reaction
PCR-RFLP	Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
PD	Postoperative Delirium
PG	Propofol Glucuronide
POCD	PostOperative Cognitive Dysfunction

PONV	PostOperative Nausea and Vomiting
PQRS	Postoperative Quality of Recovery Scale
Q	Quinol
QoR 40/15	Quality of Recovery 40/15
RM ANOVA	Repeated Measurement Analysis of Variance
ROC	Return of Consciousness
SNP	Single Nucleotide Polymorphism
TCI	Target Controlled Infusion
UDP	Uridine DiPhosphate
UGT	Uridine diphosphate GlucuronosylTransferase
V_d	Volyme of Distribution

INTRODUCTION

We are all different.

The hospital was big, white, shiny and newly built. Minna and Hedda, two young women unknown to each other, were sharing a room. Beforehand they had both been promised their own spacious room with a beautiful view over the city, but unfortunately, the ward was out of single rooms, too many patients had been admitted to the hospital in the past few days as a result of the sudden black ice that had struck the city. Both Minna and Hedda were victims of the black ice, they had broken bones and they were waiting for surgery the following day.

Minna was not really interested in talking to anyone, but her new roommate Hedda was an energic talkative person.

"Have you had surgery before?" Hedda asked, lots of excitement in her voice, and without waiting for an answer she continued:
"I haven't but I have heard from friends that getting anaesthesia is one of the best things they've ever had. It's like having a really great dream. And afterwards you're just happy. And get snacks and drinks at the postoperative ward. And then you go home and get to stay at home for a couple of days even though you feel fine, some extra days off from work for free. I have lots to do at home, the timing of my broken leg is perfect."

Minna turned to her roommate.

"I haven't had surgery before, but all my friends who have had surgery have felt terrible afterwards, throwing up for days and not been able to get out of bed for a week. To conclude, I am not looking forward to tomorrow."

Hedda stared at Minna for a short while, shrugged her shoulders and went back to the book she was reading.

Shortly after, the surgon, Dr Johanna, was entering the room.

"Good evening, hope you feel alright. Tomorrow at this time you will be newly operated and happy, any questions?" Before Minna and Hedda had time to open their mouths, Dr Johannas' phone rang, she went out to take the call and didn't come back.

Minna spent the rest of the evening trying to solve the crossword from "Svenska Dagbladet", but she was unable to concentrate, all her thoughts were mixed up and the focus all gone. She couldn't get rid of the nagging unease she felt when thinking about tomorrow's anaesthesia and surgery.

The following day Minna and Hedda were transported to the operating ward. In the operating room, before anaesthesia was started, the anaesthetist asked a few questions about general health, former surgery, if they were ever sea sick, had any allergies and so on.
"Everything is perfect!" the anaesthetist said, "now just breathe deeply in this mask and you

will soon fall asleep." They were both given the standard doses of the intravenous anaesthetic propofol. "It's an amazing drug", the anaesthetist thought by herself," I just give 2.5 mg/kg of this terrific whitish liquid, my patient falls asleep, the infusion goes on during the surgery, I switch it off when surgery is over and my patient wakes up. I can just sit and watch and have my coffee. I have a wonderful job". Minna and Hedda went through identical surgery. The length of surgery was the same and since they were of the same size they got about the same dose of painkillers at the end of the surgery, morphine and paracetamol, as usual. The anaesthesia and surgery was uneventful with no complications. However, the schedule of the day was a little bit delayed due to the fact that it took more than 30 minutes to get Hedda to wake up. A little bit irritating the anaesthetist thought.

Minna opened her eyes on the postoperative ward. She felt rested. She was hungry and thirsty and immediately felt like stretching her limbs, sitting up and maybe trying to walk a few steps. She hardly had any pain at all. In the bed next to her she heard a familiar voice, Hedda. Hedda was spluttering when she was talking, but Minna could still hear that she was complaining about nausea. And pain.

A few hours later, Minna went back to the ward where she had a shower and read yesterday's newspaper. She had a glimpse at the crossword again and was a little surprised;

How could I not finish this off yesterday, it is simple, she thought for herself and filled in all the missing words in a few minutes.

Next she had a surprisingly well tasty dinner. They really had improved the food at the new hospital. In the evening, she spoke to Dr Johanna, who was very content with the operation. Minna asked where her roommate was and Dr Johanna chuckled;

"I guess she was a little bit weaker than you, she stayed on the postop ward over night!"

Hedda didn't showed up until lunchtime the next day. Minna had packed her belongings and was just about to leave the room and the hospital when Hedda came back from the postoperative ward. She looked pale and sick.

"Eh, hi. I am just leaving. If you haven't had lunch I can really recommend the smoked salomon, it's great!" Minna said.

Hedda lifted the plastic bag from her lap to her face. Minna plugged the headphones into her ears, increased the volume of the music and left the room. Finally Hedda got her own spacious room in the new hospital. That was a good thing since she had to stay for another three days due to nausea and dizziness. Had she been able to eat and walk she would have been impressed by the quality of food and the beautiful city view.

Minna and Hedda are both young women with the same ethnic origin. They have similar injuries and are undergoing similar operations. They are healthy and do not take any medication. In the hospital, they get similar treatment and the same drugs in similar doses. The length of their operations is about the same. Still their recoveries after surgery and anaesthesia are completely different – Minna who was worried and maybe even a little

cognitively affected the day before the surgery, is feeling well at once and can leave the hospital the following day while Hedda has to stay for several days.

This happens every day in the hospital – the recovery and resumption of mental and physical capacities after surgery and anaesthesia vary considerably between individuals. Often we don't know why. There are several factors that may cause variations in postoperative recovery, i.e. co-morbidity[1] age, gender[2,3], type and length of surgery and anaesthesia[1,4], pain[5], nausea[6], genetics[7,8].

There are remains of anaesthetics in the body for hours to days after administration[9,10]. The clearance of drugs depends on their distribution, metabolism and elimination. Most drugs are metabolized and eliminated mainly as inactive metabolites. The halogenated inhaled anaesthetics are eliminated as intact active molecules washed out by exhalation, while the anaesthetics administered by the intravenous route are usually dependent on liver metabolism for the elimination and subsequent cessation of effect. There may also be some neurochemical residual central nervous system (CNS) effects.

Propofol is one of the most used intravenous anaesthetics in the western world. Due to its pharmacokinetic properties resulting in quick onset and short emergence time it is a suitable drug for ambulatory as well as prolonged surgery. However, there are great interindividual differences in propofol pharmacokinetics and pharmacodynamics, and the reasons for this are not fully clarified[11,12]. Propofol is metabolised by highly polymorphic enzymes, which means there are more or less efficient variants in the population**.**

It is not known if a variation in the efficiency of these enzymes has a clinical significance. Variability in enzyme capacity may alter the metabolism of propofol, in some cases slow elimination and possibly prolong recovery or increase side effects.

Ambulatory surgery is increasing, and with that the patient´s length of stay in the hospital is shortened. Many patients are expected to return home the same day or the day after surgery, which requires a quick recovery with a minimum of complications. Cognitive recovery is an important part of the recovery process, during which the patient regains his/her memory, verbal ability, perception, attention, executive function and abstract thinking. It is believed that cognitive impairment observed during the first postoperative week is possibly associated with residual effects of anaesthetics and/or analgesic drugs[13-15].

It is not known whether interindividual differences in metabolic capacity of propofol contribute to variations in cognitive recovery.

The focus of the present thesis was the impact of genetic variability on the metabolism of propofol, and the variation in cognitive recovery after ambulatory anaesthesia with propofol, using the least soluble and most inert inhaled anaesthetic desflurane as control. Also the impact of preoperative stress on the cognitive test performance in patients waiting for cancer surgery was investigated.

PROPOFOL

In general

Propofol (2,6-diisopropylphenol) is an alkyl phenol which was developed in the 1970's and first described as a potential anaesthetic agent by Kay et al in 1977[16]. It has been used in Sweden since 1987 and is today one of our most commonly used intravenous anaesthetics. Propofol has favourable properties such as quick onset and quick emergence in combination with low toxicity and relatively few side effects, which makes it a suitable drug both for sedation and anaesthesia.

Propofol is mostly found to be comparable to inhaled anaesthetics with respect to time to discharge from the hospital, and often considered to decrease the occurrence of PostOperative Nausea and Vomiting (PONV)[6,17,18]. The recovery assessment after propofol anaesthesia has frequently focused on the early part of the recovery process, emergence from anaesthesia, resumption of stable vital functions and protective reflexes and further logistic factors such as "eligible for discharge" which involves regaining physical abilities such as standing, walking, and eating in order to be home ready. The more protracted recovery process after propofol anaesthesia such as resumption of cognitive capacity and becoming "street-fit" is less well defined[6,17].

Pharmacokinetics and pharmacodynamics

Propofol is highly lipophilic, which results in a rapid crossing of the blood-brain barrier, reaching the effect site and leading to a quick onset. The effect duration is short due to as quick redistribution from the brain to surrounding tissues. The pharmacokinetics are described as three compartmental; initial distribution into the central compartment, further rapid redistribution into both well and less well perfused compartments with slow redistribution back into the central compartment to be metabolised[19-21]. The slow redistribution in combination with quick metabolism adds to the quick emergence. The plasma concentration profile following a bolus dose is described in figure 1. The distribution half-life after bolus is approximately 2-4 minutes, and the volume of distribution (V_d) has been found to vary between 209-1008 l[22]. Clearance depends on whether bolus or infusion is given and varies between 77-139 l/min[21]. Context sensitive half-life up to eight hours is less than 40 minutes[23].

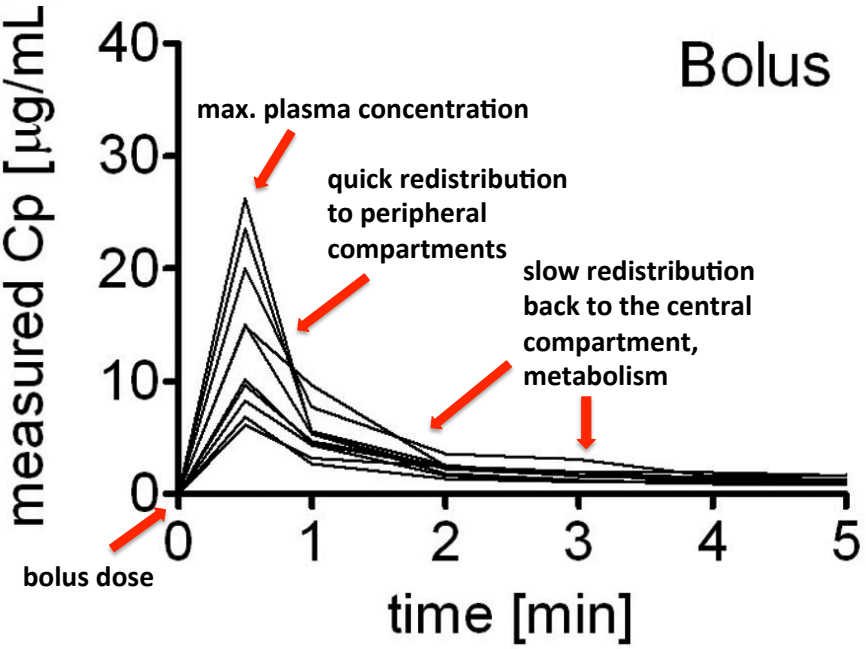


Fig. 1 The figure demonstrates the propofol concentration in plasma after bolus dose in healthy volunteers. The initial high peak concentration after injection decreases as propofol quickly redistributes into peripheral compartments, thereafter follows a slow decrease in plasma concentration, as propofol redistributes back into the central compartment and is metabolised. Masui et al 2010[24]

Propofol is metabolised by enzymes, mainly in the liver, and metabolism is almost complete, leaving less than 1 % of the drug to be excreted unchanged in the urine[10]. During anhepatic surgery such as liver transplantation, there has been evidence of extrahepatic metabolism, which is most likely taking place in the kidney[25 26 27 28]. Metabolic capacity is dependent not only on the enzymatic capacity but also on the liver blood flow and oxygenation. Thus, metabolism may be influenced by circulatory changes caused by anaesthesia itself and also by surgery[29-31]. Propofol has a high level of protein binding (96-99%) to serum albumin[32].

The metabolism of propofol follows two main routes; direct glucuronidation forming propofolglucuronide (PG) or by hydroxylation to form quinol (Q). Quinol is further conjugated with glucuronic acid or sulphatic acid to form quinol-1-glucuronide (1-QG) and quinol-4-glucuronide (4-QG), or quinol-4-sulphate (4-QS)[9,33]. The water soluble PG, 1-QG, 4-QG and 4-QS are excreted in the urine (Figure 2). PG is considered to be the main metabolite even though there is a great interindividual variation (10-67%)[9,10,33,34].

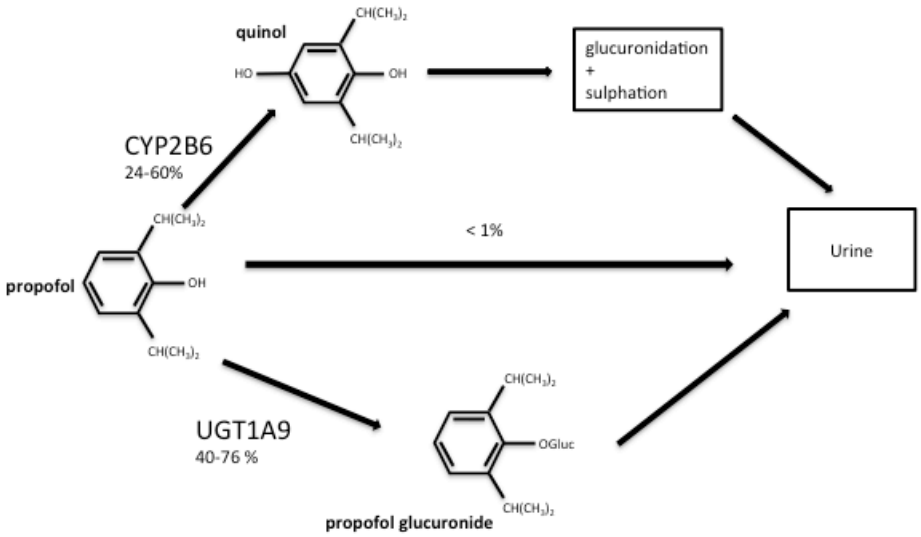


Fig.2 The figure illustrates the metabolic pathway of propofol through hydroxylation by CYP2B6 or glucuronidation by UGT1A9. The percentage stated below the respective enzyme illustrates the variation in metabolic pathway between individuals observed in several studies[10,34].

Propofol metabolites are found to remain for a long time in the body. Favetta et al. found that only 38 % of the drug had been excreted in the urine 24 hours after a bolus dose, and 60 hours after a 4 hour propofol infusion, the excretion in the urine was still not complete (30-94 %)[10,34]. The excerted metabolites glucuronides and sulphates, are pharmacologically inactive but quinol is thought to have one third of the clinical effect of propofol[35]. Thus, trace concentrations of quinol may possess pharmacological a residual effect.

Propofol is known to have brochodilatating properties[36,37], be anticonvulsive[38] and decrease intracerebral bloodflow and intracranial pressure[39,40]. Propofol possesses anti-inflammatory activity[41] and have shown antioxidative effects[42,43]. It also has antiemetic properties[6 17]. The side effects are few and relatively mild. Propofol decreases blood pressure[44] due to decreased sympaticus tonus, but cardiac inotropy is unchanged with therapeutic doses[45]. As for other intravenous anaesthetics transient apnea after induction is common[46,47].

Propofol main site of action is through stimulation of the postsynaptic inhibitory γ-aminobutyric acid (GABA) receptor $GABA_A$ and thereby increasing transmission[48,49]. Other sites of action discussed are the depressive effect on the excitatory N-Methyl 1-D-Aspartate (NMDA) receptor[40] and also stimulation of the inhibitory glycine recptor[50].

A rare but fatal complication of propofol administration is ”propofol infusion syndrome”, which has mostly affected pediatric and adolescent patients who have received the drug in high dose for many days. The syndrome seems to be caused by impaired mitochondrial respiration which in turn results in critical rhabdomyolysis, metabolic acidocis and hemodynamic collapse[23].

Genetics

The enzymes responsible for the biotransformation of propofol to its metabolites are mainly uridine diphosphate (UDP) glucuronosyltransferase 1A9 (UGT1A9) and cytochrome P450 2B6 (CYP2B6)[35,51]. Both UGT1A9 and CYP2B6 are highly polymorphic which means they exist in many variants in the population. Polymorphism may give rise to more or less efficient gene products, in this case more or less efficient enzymes, and this in turn may affect the efficiency of the metabolism[52]. It is believed that polymorphism in commonly drug metabolising enzymes may be a considerable reason for over- and under-treatment of many patients which in turn leads to an increased frequency of adverse effects, prolonged stay in hospital or a lack of effect[53].

UGT1A9 and CYP2B6 metabolism of propofol has shown to vary extensively between individuals[10 34,35]. So far, 38 alleles (variants of the same gene) of CYP2B6 have been found (www.cypalleles.ki.se). *In vivo* studies have shown increased activity for the allele CYP2B6*4 and decreased activity for the allele CYP2B6*6[54,55]. As for UGT1A9, 27 alleles are registered (www.ugtalleles.ulaval.ca). UGT1A9 M33T (*3) has demonstrated reduced propofol glucoronidation activity, while UGT1A9 with a mutation in the 275A/-2152T regions has been identified with increased propofol glucuronidation activity[56]. The frequency of the mutations varies in between ethnical groups, which is of importance for the possible clinical impact of the polymorphism in various populations[57,58].

DESFLURANE

Desflurane belongs to the third generation of halogenated inhaled anaesthetics together with sevoflurane. Desflurane is the halogenated inhaled anaesthetic with the lowest blood and fat solubility and thus the most favourable, rapid pharmacokinetics. The blood gas coefficient of about 0,42 promotes quick equilibration during induction, and the low fat, tissue and blood solubility is associated with a rapid elimination after cessation of administration. Desflurane undergoes a minimum of metabolism and thus elimination is solely dependent on exhalation at end of anaesthesia. When compared to other anaesthestics, desflurane has shown to result in the quickest emergence recovery[6,59,60]. Desflurane may thus be seen as the anaesthetic with the least variability in kinetics.

ANAESTHESTICS AND GENDER

Men and women are physiologically and pharmacologically different in numerous ways which affect the response to drug treatment. Physiological differences such as body constitution leading to different volume of distribution[2], hormonal variations affecting bioavailability[61], level of protein binding as well as cardiovascular[62] and respiratory function[63] are described. For some drugs the pharmacokinetics differs up to 40 % in between men and women[64]. Gender differences in drug metabolism is believed to be one of the main reasons behind the divergent drug reponse[65]. An increased activity for CYP3A4 is reported for women[66], and studies of human liver tissues have shown that women tend to express higher levels of CYP2B6 in liver than men[67]. However, Ilic et al. did not observe any gender difference in *in vivo* CYP2B6 activity correlated to metabolism[68]. As for anaesthetic drugs, women are more sensitive than men to the water soluble neuromuscular

relaxants due to smaller volume of distribution and thereby higher concentration in plasma[2]. Women are also found to be more sensitive to opioids than men[69]. Considering the many differences in drug response reported, it is plausible that gender difference in response to anaesthetics varies in many other ways than the above mentioned[70]. Female gender is a risk factor of PONV[71,72] and sex hormones are believed to be contributory to this difference[71-74].

Propofol and gender

Women have a higher volume of distribution of propofol and also a higher clearance than men[75], and they need a higher dose in order to maintain a preset depth of anaesthesia according to Bispectral Index (BIS) electro encephalography (EEG) analysis[76]. Women also seem to undergo a more rapid decline in propofol concentration[77] and wake up faster than men [3,12,78-80]. In one study, men were more easily sedated than women[81]. Results on gender difference in central sensitivity to propofol are contradictory[76,77].

POSTOPERATIVE RECOVERY

The postoperative recovery is a continuous process starting from the end of anaesthesia and ending when the patient has returned to his or her full preoperative status. According to the society of ambulatory surgery, recovery can be categorized into three parts; early recovery which is the period from the end of anaesthesia until the patient regains basal protective reflexes and motor movements, intermediate recovery during which the patient gets ready for discharge from the hospital, and late recovery, a period when the patient returns to the preoperative status. Previously, recovery was measured by postoperative morbidity and mortality. Today, both morbidity and mortality are relatively rare side effects of ambulatory surgery and anaesthesia, and the assessment of recovery is increasingly focusing on resumption of preoperative capacities, ability to cope with basic functions and patient´s satisfaction. The "quality of recovery" is frequently assessed, meaning the patient's subjective view of recovery.

Multiple factors affect recovery, PONV being the side effect most frequently resulting in readmittance to hospital[82]. Postoperative pain is also a common side effect which may prolong hospital stay[83]. Other pre- and postoperative factors that should be taken into consideration influencing postpoperative recovery are co-morbidity, age[84], but also preoperative anxiety and sleep deprivation[85], the last two affecting in particular the cognitive recovery of the patient. Mild residual cognitive effects are not uncommonly experienced during the intermediate postoperative course[86,87]. Both long-term and short-term cognitive dysfunction are commonly seen after surgery and anaesthesia[4,88].

Neurocognitive side effects

Neurocognitive side effects following surgery and anaesthesia have been observed and studied for many years, initially in patients undergoing cardiac surgery[89], but also after non-cardiac surgery[88]. Old age[4,90], low level of education[90], co-morbidity[4] and increased length of surgery and anaesthesia[1,91] are some of the risk factors associated to the development of postoperative cognitive impairment. There are various types of neurocognitive side effects; emergence agitation, emergence delirium, Postoperative Delirium (PD) and the more long lasting PostOperative Cognitive Dysfunction (POCD). Emergence agitation is usually seen

within hours[92] and PD 24 – 72 hours after end of surgery and anaesthesia[93]. The focus of postoperative cognitive research has mainly been on POCD, a state that refers to a prolonged postoperative change in cognition emerging after the first postoperative week and tested by extensive and sophisticated neuropsychological tests[94]. The test battery developed by the International Study on PostOperative Cognitive Dysfunction (ISPOCD)[90] is the gold standard, but since a definition of POCD is lacking and the design and cognitive tests used within the studies is varying, the results are difficult to compare[95].
Neuroinflammation as a stress response to surgery and anaesthesia is the mechanism believed to be involved in the development of postoperative cognitive impairment[96]. Whether neuroinflammation is involved in both short-term transient cognitive impairment and long-term cognitive dysfunction is not known. Jildenstal et al. found that less deep anaesthesia titrated by Auditory Evoked Potential Index (AAI) was associated with lower levels of the inflammatory interleukin IL-6 and less cognitive impairment in the first 24 hours but not one week postoperatively[97], which could indicate that an inflammatory process is being responsible for short-term cognitive impairment as well.

Postoperative recovery and anaesthesia

Anaesthesia is a pharmacological dose-dependent depression of the CNS. Awakening and CNS recovery take place after cessation of drug administration and is related to the elimination of anaesthetics and residual pharmacodynamic effects. It is difficult to differentiate between the natural cognitive recovery following surgery and anaesthesia and a possible mild neuro-inflammatory insult. Difficulty in the ability to concentrate during the first postoperative hours to days[5 98], and transitory residual cognitive decline resolving during the postoperative first week is commonly reported[99]. Most clinical studies on postoperative cognitive impairment are conducted from one week postoperatively, and focus on the development of POCD. There is no consensus on when postoperative cognitive impairment is really POCD, but in a review by Newman et al., all POCD-studies with neuropsychological assessments within one week after surgery were excluded in order ”to avoid the general effects of any anaesthetic agent”[88], and in several studies it is suggested that the cognitive impairment within a week after surgery and anaesthesia is caused by remaining anaesthetic and analgesic drug effect rather than a pathological structural change in the CNS[13-15]. Royse et al. compared cognitive recovery after propofol or desflurane anaesthesia in patients undergoing coronary artery bypass, and found a worse cognitive recovery in the propofol group at day 3-7 postoperatively but no difference at three months postoperatively[100]. It is from those results not possible to assess whether these differences were caused by remains of anaesthetics or remaining effects on the CNS by anaesthetics or by a systemic stress response including neuro-inflammation.

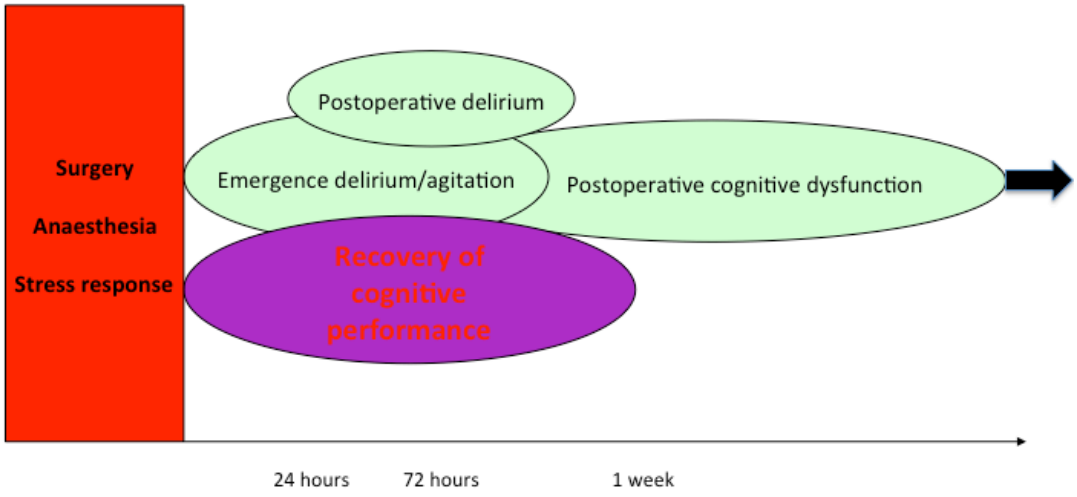


Fig. 3 The figure demonstrates when the postoperative cognitive recovery and pathological neurocognitive side-effects after surgery and anaesthesia usually occur. Emergence delirium and agitation and postoperative delirium normally cease within one week postoperatively. POCD, PostOperative Cognitive Dysfunction is believed to develop from one week postoperatively and onwards. Recovery of cognitive perfomance back to preoperative status is expected to start immediately postoperatively.

Postoperative cognitive recovery and regional vs. general anaesthesia

Anaesthesia has its site of action in the brain and it is known to affect memory and learning[101,102]. It is possible that remaining impact of anaesthetics cause transient postoperative cognitive effects in patients. This is strengthened by the fact that general as opposed to regional anaesthesia is associated with a greater risk of short term cognitive dysfunction[13-15]. Local and regional anaesthesia is associated with a more favourable early and intermediate recovery[103-106].

Several studies demonstrate better cognitive performance day 1 – 3 after surgery among patients who received regional vs. general anaesthesia[107,108]. However, there is still no clear evidence to show whether there is a difference in the risk for neurocognitive side effects between general and regional anaesthesia[13,109]. A meta-analysis from 2010 concluded that it appears that general anaesthesia, compared to others, may increase the risk of developing POCD; however this has not been shown for PD[110]. The impact of depth of anaesthesia on postoperative cognitive impairment is controversial with opposing results[111-113].

Postoperative cognitive recovery and choise of general anaesthetic

Early cognitive recovery with respect to different anaesthetic regimens has been studied, but cognitive test methods, type of surgery and anaesthetic care all vary. Larsen et al. found that anaesthesia with propofol and remifentanil resulted in a quicker cognitive recovery

than desflurane or sevoflurane[114], but Biedler et al. using a more complex cognitive test battery, found the opposite[115]. Moore et al found no difference in 4 different anaesthetic regimens when asking for ”forgetfulness” and problems to concentrate up to one week postoperatively[98]. Cognitive dysfunction was as high as 46 % 24 hours postoperatively in an group of elderly patients undergoing general surgery, no difference between sevoflurane and propofol anaesthesia was seen[116]. Parida et al. observed that early cognitive function was slightly quicker after sevoflurane compared to propofol[117], but that home readiness was similar between groups.

Assessment of Postoperative Recovery

The recovery assessment tool to be used depends on which part of recovery is to be evaluated; early, intermediate or late recovery**.** Former assessment tools have focused on just one phase in recovery such as the Aldrete score[118] evaluating early recovery and the Post-Anaesthetic Discharge Scoring System (PADSS) assessing intermediate recovery[119]. Recently the Postoperative Quality of Recovery Scale (PQRS) was developed, covering all three phases of recovery[120].

The assessment of recovery of today is a multidimensional judgement based on various parameters such as physiological, cognitive and emotional parameters. Various tools for assessment of quality of recovery have been developed and used, which makes comparison between studies difficult. In 2000, Herrera et al. found that Quality of Recovery 40 (QoR40) at that time was the only recovery instrument for ambulatory surgery that fulfilled the criteria from a quality point of view. However, this tool was considered to be inappropriate for ambulatory surgery[121]. QoR40 was shortened to develop QoR15 which has also been validated[122]. Both QoR 40 and 15 are tools asking for the patient’s subjective view of recovery. The Cognitive Failure Questionnaire (CFQ) is also a patient’s subjective assessment tool, purely cognition focused. The CFQ was initially used within the field of psychology, but then used to assess postoperative cognitive recovery[123,124].

Objective assessment of postoperative cognitive recovery

Cognitive recovery assessment is complex. Quality of recovery tools are important to reflect the patient’s view of recovery. However, it has been repeatedly shown that the patient’s subjective view of cognitive recovery is usually not consistent with objective cognitive recovery. Therefore objective cognitive tests are of great importance[125,126]. In year 2010, the Postoperative Quality of Recovery Scale (PQRS) was developed, a multidimensional tool involving six domains of importance for recovery (physiological, nociceptive, emotive, activities of daily living, cognition and the overall patient perspective)[120]. The scale has been validated for various patient groups and surgical interventions[86,127], and is intended to be used for both early, intermediate and late recovery after surgery and anaesthesia. As opposed to most other recovery scales, which have a composite score, the PQRS has dichotomous score for each domain, making it easier to identify within which area the patient is recovered or not recovered. Also, the PQRS cognitive domain consists of five validated objective neurocognitive tests while most other recovery tools assess subjective cognitive recovery[128]. However, it is stressed that the cognitive domain of the PQRS is not to be compared with the more complex neurophysiological tests battery intended to diagnose POCD, but instead designed to evaluate the patient’s return of cognitive function after surgery and anaesthesia[129].

In summary, ambulatory surgery and early postoperative discharge from the hospital is increasingly adopted. This demands a quick and smooth postoperative recovery. Cognitive recovery is an important part of the recovery process, not the least for patients undergoing ambulatory surgery. Impaired cognitive recovery within the first week after surgery and anaesthesia can be partly caused by remaining trace concentrations of the anaesthetic drug and/or its possibly active metabolites, or by residual effects in the CNS per se.

The postoperative recovery varies a lot in between patients, and the factors behind this are not fully clarified. Propofol is often used in ambulatory anaesthesia, due to fast onset and short emergence. The pharmacokinetics and pharmacodynamics of propofol vary a lot in between individuals and the reasons for this are unknown. Propofol is metabolised by polymorphic enzymes, and there are more or less efficient variants in the population. It is not known whether this polymorphism has a clinical impact on the metabolism and as a result of that on the early as well as the intermediate postoperative cognitive recovery.

AIMS

The overall aim was to study propofol with respect to genetical variations and metabolite production, and furthermore to investigate the interindividual variations in cognitive recovery after ambulatory surgery with propofol anaesthesia.

The specific aims were:

- To study the effect of genetic polymorphism in CYP2B6 on propofol hydroxylation capacity in human liver microsomes.

- To study the effect of genetic polymorphism in propofol key metabolising enzymes CYP2B6 and UGT1A9, age and gender on the production of propfol metabolites in vivo after propofol bolus and infusion.

- To assess the cognitive recovery using the PQRS and CFQ cognitive tests during the first postoperative week after ambulatory breast cancer surgery comparing propofol and desflurane anaesthesia.

- To study the PQRS cognitive, nociceptive and emotional test performance at test re-test in pre-surgery breast cancer patients compared to controls.

MATERIALS AND METHODS

ETHICAL CONSIDERATIONS

All studies were approved by the local ethical committee in Stockholm. All the participants in the studies signed an informed consent and were informed of the possibility to leave the study at any time point.

PAPER I-II

Livers

For the *in vitro* part of paper I, livers from earlier established liver banks at Karolinska Institutet were used. The liver banks were approved by the Regional Ethical Committee. In the study, liver material from 68 different livers were used, 32 from men, 34 from women and 2 from unknown gender.

Patients

The patients included in study I and II were scheduled for minor elective orthopaedic hand and foot surgery. They had a physical status classified according to American Society of Anesthesiologists I-II (ASA I-II). None of the patients had any cardiovascular, kidney or liver disease, nor were taking any regular medication. In study I, a total of 105 patients were included, 80 women and 25 men between 16 and 76 years of age. In study II, 98 patients were included, 53 women and 45 men with a median age of 48 years.

In vitro study

Analysis of CYP2B6 content and associated propofol metabolite production was performed according to the method described by Court et al[130]. 100 μg of human liver microsome, NADPH and propofol was allowed to react for 10 minutes, and then stopped with acetonitrile. The level of CYP2B6 was analysed through Western Blot; the proteins were separated through gel electrophoresis, standards of different CYP2B6 concentrations were added to each gel. The proteins were transferred onto a membrane, thereafter anti-human anti bodies were added as well as goat anti-rabbit IgG with horse radish peroxidate (HRP). An enhanced chemoluminiscent kit detected the chemoluminiscent reaction which correlated to the amount of CYP2B6, and the levels of CYP2B6 were calculated using Image Gauge V4.0 software (Fuji film).

Anaesthesia

In both paper I and II, the patients had peroperative monitoring with electrocardiography, non invasive blood pressure, pulse oximetry and capnography. Before induction of anaesthesia, 8 mg of betamethasone was given (as a prophylactic antiemetic). In paper I, with an expected time of surgery of 15-20 minutes, anaesthesia was induced with a bolus dose of propofol until Loss Of Verbal Response (LOVR), and alfentanil of 0,4 μg/kg. Anaesthesia was maintained with inhalation of sevoflurane and oxygen (FiO2 0.5). Ventilation was spontaneous and assisted when necessary. Blood samples were taken before induction, 4, 8, 12, 16 and 20 minutes after induction.

In paper II, with an expected time of surgery of approx. 30 minutes, the induction of anaesthesia was performed with alfentanil 2-3 μg/kg and a Target Controlled Infusion (TCI, Alaris Marsch model) of propofol with a calculated effect site concentration of 4-5 mg/L. The patients were breathing spontaneously through a laryngeal mask a mixture of oxygen and air (FiO2 0.4). Blood samples were taken before induction, 10, 17, 21, 25, 40 and up to 90 minutes after induction.

Blood samples

Blood samples were stored at 4°C for a maximum of 4 hours before they were centrifuged. After centrifugation, buffy coat (leucocytes) was separated and stored in -20 °C for later genetic analysis. Further, plasma was separated, acetonitrile with standard thymol was added to stop any ongoing metabolism, and the samples were vortexed and centrifuged. The supernatant was either immediately analysed for propofol and its metabolites, or stored in -20 °C for a maximum of 7 days before analysis.

Genotyping

Genetic analysis was performed for the alleles CYP2B6 *4, *5 and *6 in the *in vitro* study, CYP2B6 *1, *4, *5, 6, *7 and *9 and UGT1A9 *3 and -275T>A/-2152 in paper I and CYP2B *4, *5, *6, *7, *8, *9, *13, *14 and UGT1A9 *3 and -275T>A/-2152 in paper II. Genomic DeoxyRibonucelic Acid (DNA) was separated from the liver microsomes using QIAamp Tissue DNA preparation kit (Qiagen) and from the human leucocyte cell using QIA amp DNA mini kit (Qiagen). The quality and quantity of the purified DNA was analysed using NanoDropUV-Vis spectrophotometer.

The Single Nucleotide Polymorphisms (SNPs) associated with the various alleles were analysed using Polymerase Chain Reaction-restriction Fragment Length Polymorphism (PCR-RFLP) for the liver microsomes[54]. For the genotyping of the human DNA, real-time PCR with predesigned Taqman allelic discrimination assay was used (ABI 7000 Sequence detection System, Applied Biosystems).

In short, DNA is denaturated, Taq polymeras binds to predesign primers in the region of the SNPs of interest, thereafter new DNA chains are constructed. The reaction is repeated for exponential production of DNA. For the real-time PCR, a probe with fluorescence binds to the region with the SNP, leading to a fluorescent reaction where the SNP of interest is present, making it possible to accurately evaluate incidence of SNPs in the material.

HPLC analysis

To analyse the plasma levels of propofol and propofol metabolites; PG, 4-OHP, Q1G and Q4G, High-Performance Liquid Chromatography (HPLC) according to the method of Court et al[130] and Vree et al[131] was used. In short, the plasma samples are injected into the HPLC, and depending on the polarity of the substances to be analysed and the polarity of the stationary (column) and the mobile (solution running through the column) phases in the HPLC, the retention time (the time taken to pass the column) varies. After having passed the stationary phase, the substances are detected via ultraviolet and fluorescent light at specific wavelenghts, and based on how much light is absorbed, the amount of each substance present can be calculated. Plasma samples were prepared with internal standards with known HPLC retention times, thymol for the analysis of propofol and 4-OHP, and hippuric acid for the analysis of propofol- and 4-OHP glucuronides.

Statistics

In order to find the amount of propofol and propofol metabolites present in each sample, the Area Under the Curve (AUC) from t=0 to last measurable point at the HPLC plasma concentration-time curve, was calculated using the trapezoidal rule (WinNonLin 5.2.1). The results were normalised with respect to body weight and propofol dose.
Statistical analysis was performed with GraphPad Prism 5 (Graphpad Software) and Stata 11.2 (StataCorp LP). Normality distribution was calculated using Kolomogorov-Smirnov test. Spearman's correlation analysis was used for CYP2B6 protein content in livers and liver metabolic activity, and also for association between total dose of propofol with continuous variables such as age and weight. Mann Whitney U-test and Kruskal – Wallis test were used to study the relation between propofol, its metabolites and genotype and gender. Any deviation from the Hardy-Weinberg equilibrium of genotype frequency was calculated using Chi square test.

PAPER III-IV

Patients and controls

Paper III: 101 ASA I-II women between 20 and 65 years of age planned for breast surgery for suspected cancer were primarily enrolled. Exclusion criteria were cardiovascular, liver or renal disease, cognitive or psychiatric disorder or lack in Swedish fluency. The surgery was planned within the coming three weeks, expected to last 60-120 minutes and consisted of sector resection, partial or total mastectomy. 37 patients were subsequently excluded due to various reasons, and in the end 64 patients were participating in the study. Patients were divided into age groups; 20-45 years and 46-64 years, and within these groups, patients were randomly allocated to get propofol or desflurane as the primary anaesthetic drug. The age division was performed to avoid a skew distribution between age and anaesthetic drug. The patients were blinded to what anaesthetic was given, but not the research personnel due to limited resources.

Paper IV: 71 subjects were initially included in the study; 33 ASA I-II patients and 38 healthy controls. One patient and eight controls were excluded before baseline test, mainly due to emotional instability, and another patient was lost to follow up. The inclusion criteria for the patients were 20-65 years of age and planned for breast surgery for suspected cancer within the coming three weeks but at least 10 days away from the inclusion day. The controls were in the same age span, all working at Karolinska University hospital, and had no planned surgery ahead. The exclusion criteria were the same as in paper III for both groups.

Anaesthesia (paper III)

All patients had peroperative monitoring with electrocardiography, noninvasive blood pressure, pulse oximetry and capnography. The induction of anaesthesia was the same for both groups; propofol 2–2.5 mg/kg and alfentanil 0.01–0.15 mg/kg. The airway was secured by laryngeal mask placement. Ventilation was spontaneous and assisted when <8 breaths/min. PostOperative Nausea and Vomiting (PONV) prophylaxis was given in accordance to Apfel score[72]. Anaesthesia was maintained with desflurane inhalation or propofol infusion (TCI) in the desflurane- and propofol group, respectively. Bispectral

index monitoring (BIS) was used aiming at a level of BIS 40–50. Alfentanil was given perioperatively in doses of 5–7 μg/kg when signs of pain. Vital parameters and unexpected events were recorded. Emergence time, vital signs, anaesthetic, analgesic and anti-emetic drug consumption was recorded peroperatively and during the stay at the Post Anaesthesia Care Unit (PACU). Postoperative pain was treated with paracetamol 1 g every six hours, celecoxib 200 mg once daily and morphine i.v. 0.02-0.06 mg/kg if needed.

Assessment of postoperative recovery (paper III, IV)

The assessment tools used to evaluate the postoperative recovery were a modified version of the PQRS (paper III and IV) and the CFQ (paper III). We used the physiological (paper III), cognitive, nociceptive (pain and nausea) and emotive (sadness and anxiousness) domains of PQRS.

To be postoperatively recovered according to the PQRS, the postoperative score should reach baseline score or better for the nociceptive and emotive domains. For the cognitive domain, recovery was set according to the recently revised scoring system allowing for the normal variation in cognitive performance shown in healthy volunteers[127]. Beyond that, Royse et al. set a level of "good group recovery" when at least 80 % of the subjects in a group reached cognitive recovery. This was the level of "recovery" reached by the volunteer group not exposed to any event but merely performing re-tests, thus corresponding to the "healthy group normal variation" in cognitive performance. During the preparation of manuscript III, and before the article about revised scoring by Royse was published, we were informed of the forthcoming revision of the cognitive scoring change through personal communication with Royse. We did not know by then that the revised scoring system would also imply that individuals with a baseline score equal to or lower than the allowed normal variation should be excluded from the cognitive assessment. Thus the exclusion of subjects due to low baseline score was not used in paper III. However, in paper IV, the exclusion recommendation was applied. The scoring system for the CFQ implies that the higher score, the more cognitively affected. To be cognitively recovered according to the CFQ, postoperative score should be more than [baseline score +2] to allow for some normal variation in everyday cognitive performance.

Paper III: The patients performed the baseline PQRS and CFQ at the time of inclusion in the study. After surgery and anaesthesia, PQRS was performed at 2, 20 (face to face) and 48 hours (by phone) postoperatively, and CFQ was performed at 72 hours and 1 week (the patient returned the questionnaire by mail). The postoperative results were compared to the preoperative results and recovery was judged according to the pre-set definitions.

Paper IV: The patients and controls performed baseline PQRS at the time of inclusion (face to face), and re-tests at 20 and 48 hours after baseline test (by phone). Assessment was made with respect to baseline performance (number of patients excluded due to low baseline score), variability in cognitive performance as the change in score between baseline and respective re-tests, and also to the proportion fulfilling "recovery" at each re-test. The nociceptive and emotional domains of PQRS were assessed with respect to absolute score at each test and "recovery" at re-tests.

Statistics

Paper III: Differences in recovery between groups were analysed using contingency tables with Chi-square test and Fisher´s exact test. Continuous variables such as duration of anaesthesia were calculated using a two-tailed T-test. All statistics were performed using GraphPad Prism version 6.00. A p-value of < 0.05 was considered as statistically significant.

Paper IV: The number of subject fulfilling the baseline test performance in each group was analysed with Chi-square test and Fischer´s exact test. RM ANOVA was used to analyse difference in change in absolute cognitive score over time, as well as the difference in nociceptive and emotional absolute score. The within-group variation in change in nociceptive and emotional score over time was assessed using one-way ANOVA. The difference in recovery between groups for both the total cognitive domain and each cognitive question, as well as for nociceptive and emotional recovery were analysed with the Cochran Mantel Haenszel test. A $p< 0.05$ was considered significant. Based on the exclusion rate seen in other patient studies (up to 28 %), we regarded an exclusion rate of 30 % between patients and controls to be significant. With a power of 0.8 and $p<0.05$, a study size of 27 in each group was needed. Statistics were performed with Graphpad Prism version 6.0e Statsoft, La Jolla, California, USA and IBM SPSS statistics V20.0 Armonk, NY, USA.

SUMMARY OF RESULTS

PAPER I

In paper I we investigated the impact of genetic polymorphism in key metabolizing enzymes CYP2B6 (*in vitro* and *in vivo*) and UGT1A9 (*in vivo*) on propofol metabolite production after propofol bolus. *In vitro* results showed a 28.5-fold variation in CYP2B6 protein content in between liver samples (figure 1a below), with increasing amount of CYP2B6 being correlated to increased propofol hydroxylation activity. In addition, the female HLM contained more CYP2B6 protein compared to male HLM, however with no correlation to increased hydroxylation activity. In the *in vivo*-study, a 50-fold interindividual variation in propofol metabolite production was found (figure 1b below), but with no correlation between any genotype and level of metabolite. There was an increased level of all glucuronidated propofol metabolites (1.2-2.5-fold) in women compared to men.

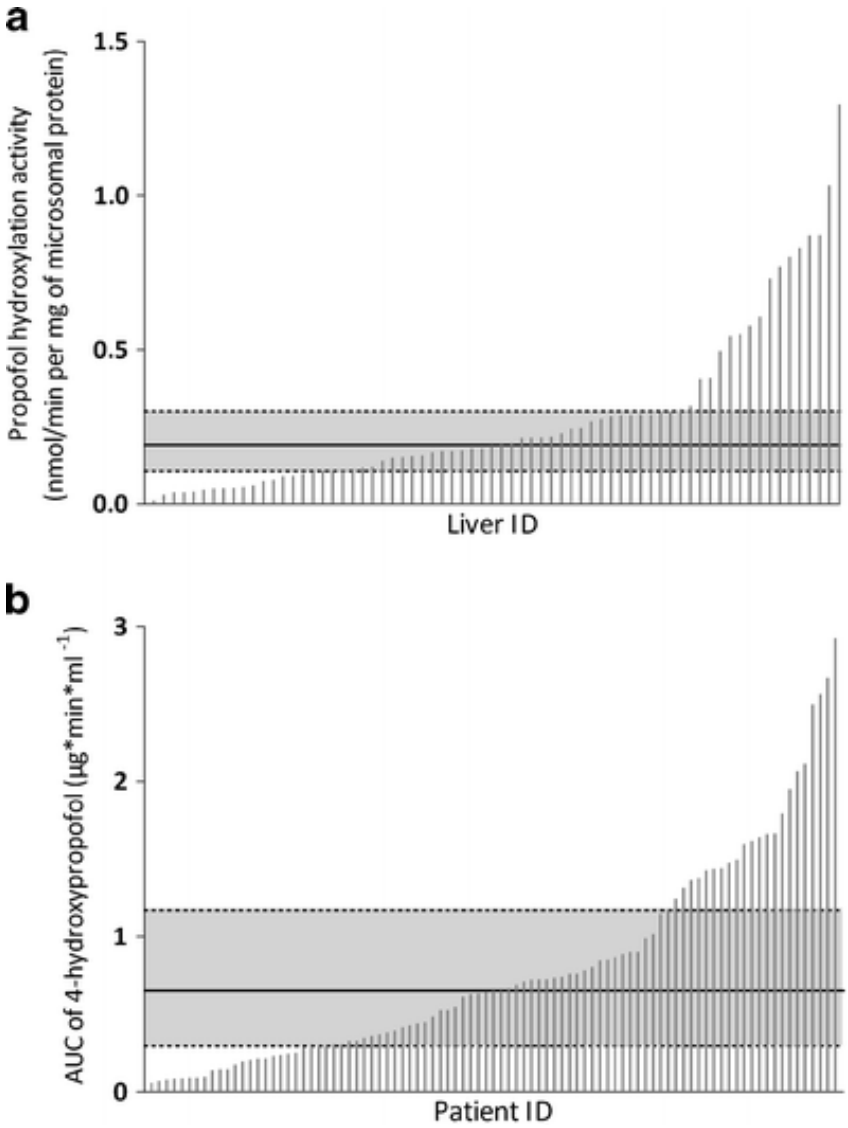


Fig.1 Formation of 4-hydroxypropofol (4-OHP) in human liver microsomes (HLM) (a) and area under the time–plasma concentration curve measured over 20 min (AUC20min) of 4-OHP (b). Bars indicate rate 4-OHP formation in HLMs at 5 µM propofol expressed as nmol/ min per mg of microsomal protein (a) and the amount in plasma expressed as AUC20min of 4-OHP µg*min*ml-1 (b). Solid lines indicate mean values and grey shaded areas indicate 95% confidence intervals (CI).

PAPER II

In paper II we wanted to validate the *in vivo* result from paper I by using a study population more evenly distributed between genders, and also to evaluate the metabolite production during more steady-state like conditions by administrating propofol as a short infusion (figure S1 below).

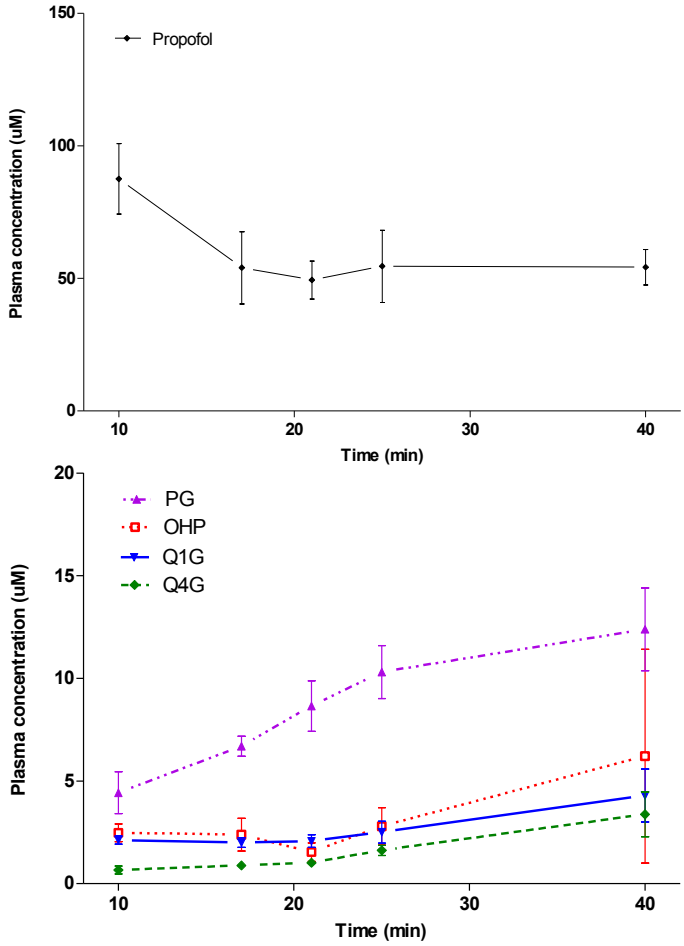


Figure S1.
Plasma concentration-time curve of propofol, 4-hydroxy-propofol (OHP), propofol glucuronide (PG), 4-hydroxypropofol-1-glucuronide (Q1G) and 4-hydroxypropofol-4-glucuronide (Q4G) after propofol infusion (mean ± standard error).

Similarly to the results in paper I, we found a considerable variation in propofol metabolites produced, but no correlation between genotype and propofol metabolite production. There was no difference in propofol concentration between gender, but a greater production of all propofol metabolites in women compared to men (1.5-4.8 - fold). A new finding in this study compared to study I, was the gender difference in CYP2B6 metabolite 4-hydroxypropofol level, where a 4.8 fold increased level of metabolites was observed in women compared to men.

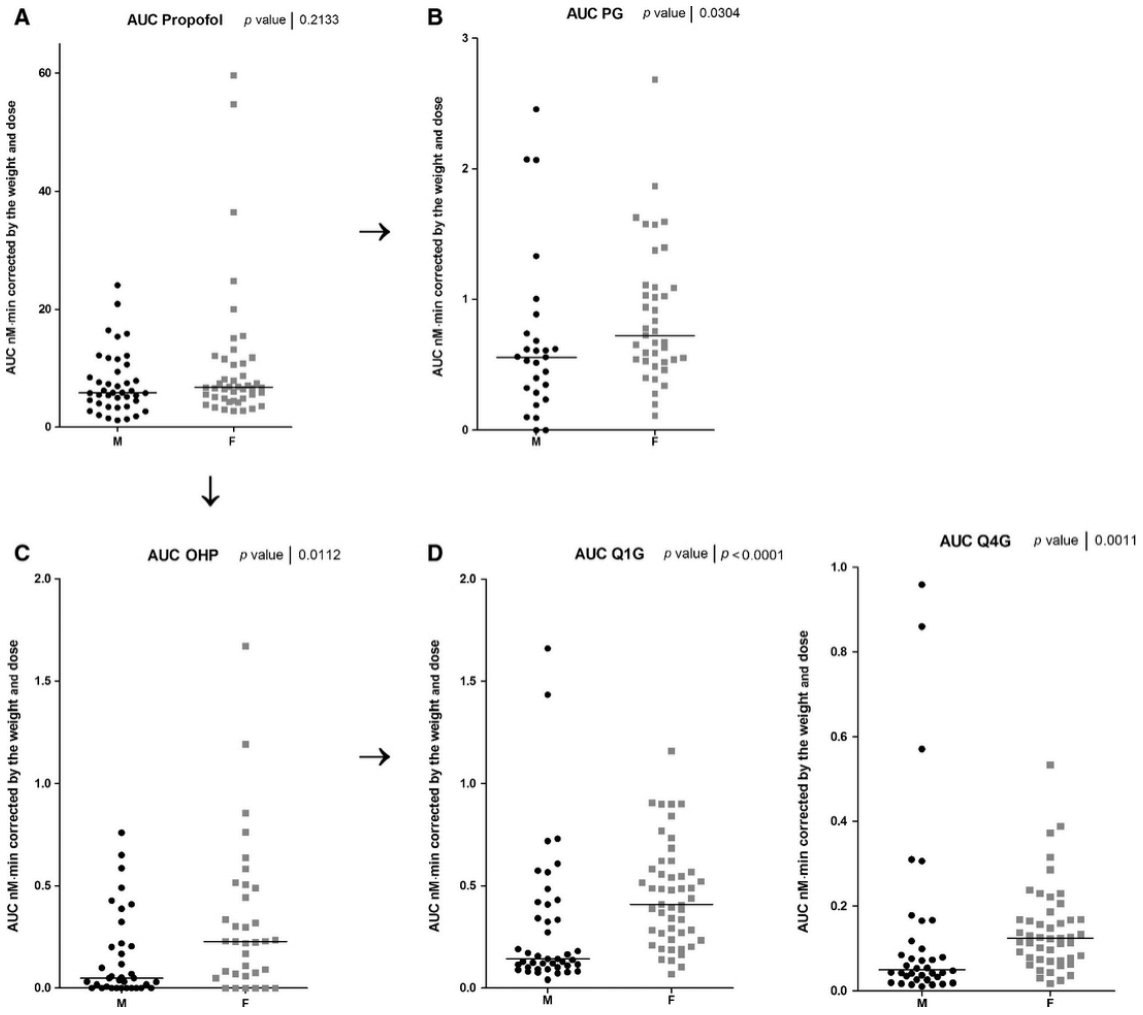


Fig.1. Sex variability of dose- and weight-adjusted area under the time plasma level curves (nM min/mg/kg) measured from 0 min. to the last time-point (AUC). A. Propofol AUC B. UGTA1 metabolism pathway: AUC of propofol glucuronide (PG) C. CYP2B6 (CYP2C9) metabolism pathway: AUC 4-hydroxypropofol (OHP) D. From OHP, subsequent UGT metabolism pathway: AUC of 4-hydroxypropofol-1-O-b-D-Glucuronide (Q1G) and 4-hydroxypropofol-4-O-b-D-Glucuronide (Q4G)

PAPER III

In paper III, the aim was to study the impact of propofol compared to desflurane on variation in subjective and objective postoperative cognitive recovery after elective ambulatory surgery. Our hypothesis was that possible remains of propofol and its metabolites would result in an impaired cognitive recovery in the propofol group compared to the desflurane group. The study groups consisted of women only, in order to avoid any impact by gender on recovery rate. We found no difference in the postoperative cognitive recovery rate between patients receiving propofol and desflurane anaesthesia according to the assessment methods used, PQRS and CFQ. We did observe a trend towards higher rate of recovery in the propofol group at 48 hours according to the PQRS, however not statistically significant. Notable was that in none of the groups, more than approx. 2/3 of the patients considered themselves cognitively recovered (CFQ) one week after surgery and anaesthesia. Patients demonstrating objective cognitive recovery at 48 hours were often not recovered according to CFQ at 72 hours and vice versa. There was also variability in the PQRS results with some patients performing better during recovery as compared to the preoperative baseline test. The recovery rates regarding nociceptive and emotional domains were similar between the groups.

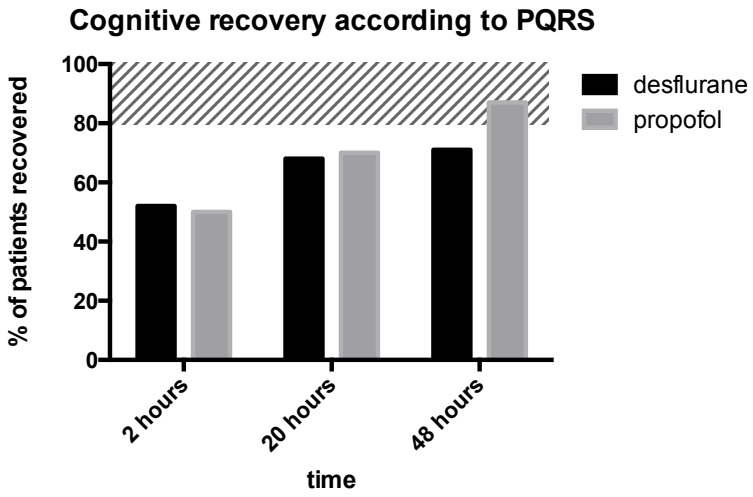


Fig. 3. Per cent of patients cognitively recovered at 2, 20 and 48 h after desflurane (black bars) or propofol (grey bars) anaesthesia according to Postoperative Quality of Recovery Scale (PQRS). The stripe-shaded field represents the area of 'good recovery' according to Royce et al. (Anesthesiology 2013). There is no significant difference between the groups at any time point.

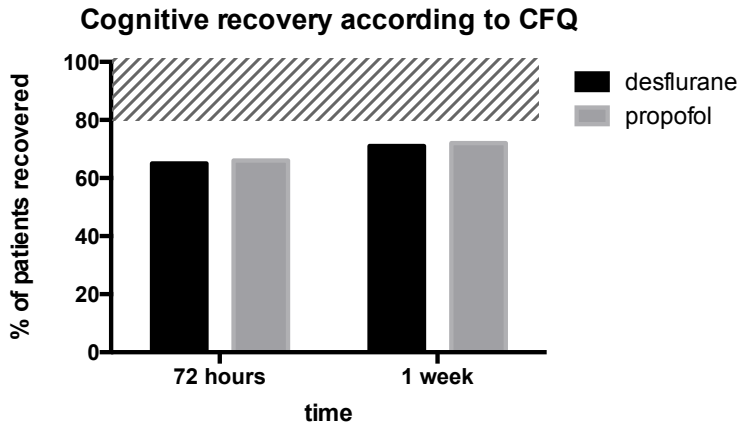


Fig.2 Per cent of patients cognitively recovered at 72 h and 1 week after desflurane (black bars) or propofol (grey bars) anaesthesia according to the Cognitive Failure Questionnaire (CFQ). The stripe-shaded field represents the area of 'good recovery', which is extrapolated from Royce et al. (Anesthesiology 2013). There is no significant difference between the groups at any time point.

PAPER IV

In paper IV, we aimed to investigate to what extent worry and anxiety due to a recent cancer diagnosis and approaching surgery could influence the baseline score and cognitive change in score at re-test using the PQRS as a recovery assessment tool. This aim was based on our somewhat surprising findings in paper III where some patients performed cognitively better postoperatively than preoperatively, which we believed could be caused by a relief that surgery was over. We found that women with newly diagnosed malignant disease expressed a higher level of anxiety than controls (fig. 4). Cognitive test performance at baseline was worse in the patient group, resulting in a significantly higher number of patients compared to controls being excluded due to low baseline score. We found no difference in the change in cognitive score at test re-test when compared to controls (fig. 2). There was no difference in the proportion of patients compared to controls being "recovered" at re-tests (fig. 3).

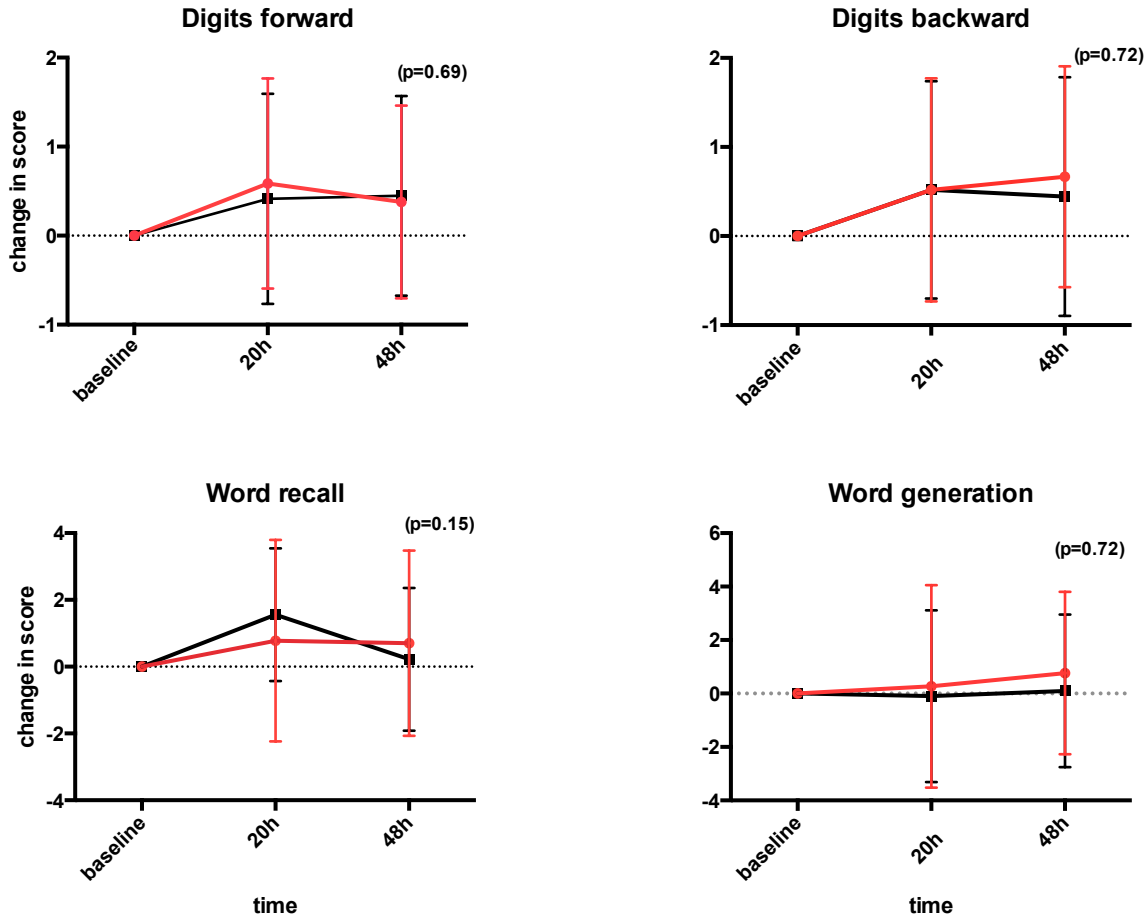


Fig. 2 The mean change in score and standard deviation (SD) for the cognitive questions of PQRS for patients (red) and healthy controls (black) from baseline until 48 hours after baseline. The "orientation" test was excluded from further analysis since all subjects scored a maximum at all test points. The error bars show ±1 SD. $p<0.05$ is considered as significant. PQRS; Postoperative Quality of Recovery Scale

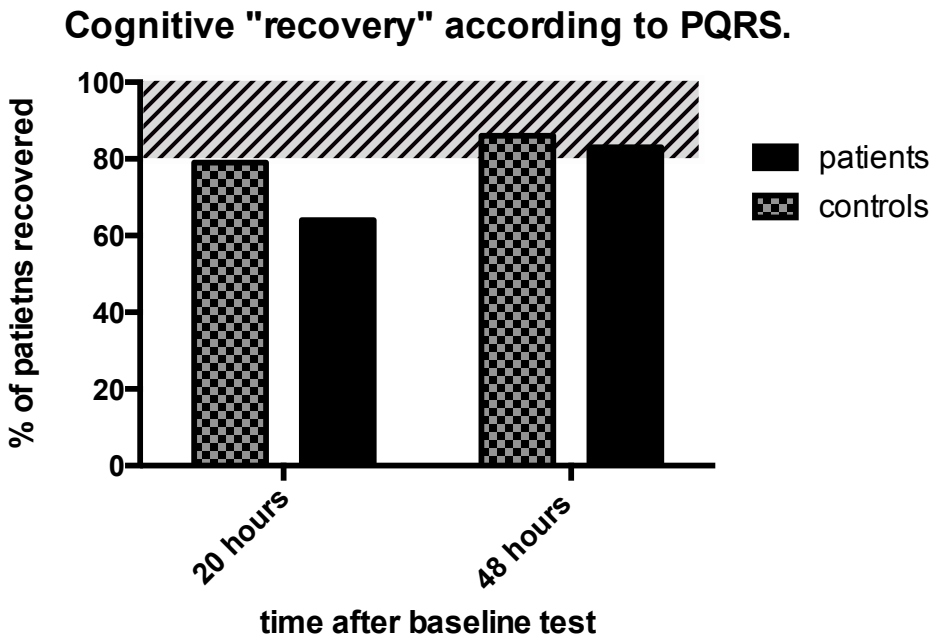


Fig. 3 The proportion (%) of patients (n=22) and healthy controls (n=28) "recovered" at 20 and 48 hours after baseline test. The stripe-shaded field represents the area considered to be the "good group recovery" (80-100%) according to Royse et al. (Anesthesiology 2013). PQRS; Postoperative Quality of Recovery Scale.

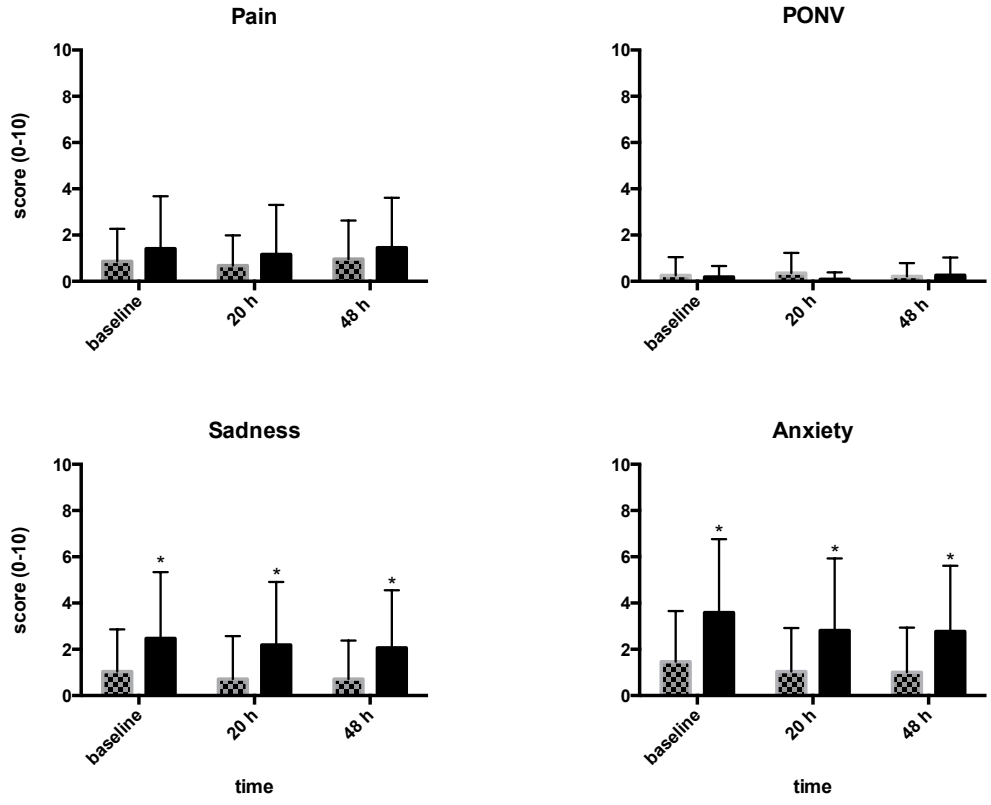


Fig. 4. The absolute score for the nociceptive (pain, PONV) and emotive (sadness, anxiety) domains of PQRS for patients (black bars) and healthy controls (checked bars) at baseline, 20 hours and 48 hours after baseline. p* <0.05 is considered significant. PONV; PostOperative Nausea Vomiting, PQRS; Postoperative Quality of Recovery Scale

DISCUSSION

In this thesis, we were aiming at investigating possible reasons for and characteristics of the interindividual variation in clinical response to propofol. Our hypothesis was that inter-individual differences in genotype could have an impact on the propofol metabolite production, which would lead to variations in residual concentrations of active substances, which in turn could delay the neurocognitive recovery. A combination of laboratory and clinical studies were performed.

We started with a pharmacogenetic study *in vitro* and then proceeded with clinical studies combined with pharmacogenetic analysis. However, due to the low expected number of individuals with rare genotypes, a clinical study was not found to be feasible. We therefore chose to design our later clinical study with a focus on investigating possible variations in postoperative cognitive recovery after propofol anaesthesia. Due to our findings in the recovery study we also studied the impact of worry and anxiety on cognitive performance in pre-surgery cancer patients.

Propofol has been subjected to various studies investigating pharmacodynamic and pharmacokinetic variations, as well as postoperative emergence and general recovery with a focus on the first 24 hours. However, to our knowledge only one former study have investigated the impact of genotype on the propofol metabolism in humans[11]. Neither has the more protracted recovery beyond postoperative day 1 – 2 with a focus on the neurocognitive recovery been extensively studied.

We found a large difference in propofol metabolite production between individuals, but no correlation to genotype. Women showed a higher metabolite production than men. The protracted cognitive recovery after propofol and desflurane anaesthesia was similar according to PQRS and CFQ, and subjectively not complete one week after surgery. Thus our hypothesis that possible residual pharmacological effects of propofol and/or its metabolites would delay cognitive recovery in comparison with desflurane was not verified. We did however a surprising finding; in both groups several patients showed a considerable postoperative improvement in the cognitive test when compared to the baseline results.

We found that the pre-surgery cancer patients had a lower baseline cognitive test performance than controls, resulting in a high number of patients not included in further cognitive testing. The patient group also expressed a higher level of anxiety. The groups had a similar test re-test performance in the PQRS cognitive domain. The emotional stress caused by a severe disease and approaching surgery should be taken into account as a confounding factor affecting the cognitive performance.

PROPOFOL AND PHARMACOGENETICS

It is well known that the pharmacokinetics and pharmacodynamics of propofol is complex and vary between individuals[12] and seemingly also in between gender[70,132]. There are most likely numerous of reasons for the interindividual variations, and since propofol is metabolised by the polymorphic enzymes UGT1A9 and CYP2B6, a genetic variance could be one of these reasons.

In study I, our *in vitro* results revealed a large variation in propofol hydroxylation capacity

between liver samples, with a correlation between the amount of CYP2B6 protein content and hydroxylation capacity. We found however, testing for CYP2B6*4, *5 and *6, no correlation between hydroxylation capacity and genotype. In the *in vivo* part of the study, we primarily studied the genotypes most associated with increased and decreased activity, CYP2B6*4 and CYP2B6*6 respectively (also *1, *5, *7, *9). In study II, CYP2B6*8, *13, *14 and *18 were analysed as well, in order to expand the search for correlation between metabolic level and genotype. As in the *in vitro* study, we found a great interindividual variation in propofol metabolite production in both study I and II, but no correlation between genotype and level of metabolites.

It is important to consider the expected frequency of the particular allele in the population to be studied. Our study population consisted of mainly Caucasians. CYP2B6*4 is generally a rare allele in the world, and existing in only 2-4 % in the Caucasian population, while CYP2B6*6 on the other hand is relatively common, found in around 25 % of Caucasians[133]. For UGT1A9 the expected frequency is around 6% for -275/2152[56] and 4 % for UGT1A9*3. The observed number of alleles in our studies was within Hardy Weinbergs equilibrium, meaning they occurred within the expected frequency in the studied population. Still, the number of individuals with the alleles was small, and a larger study population would have been desirable.

Another circumstance to consider is a possible substrate depending activity of the enzyme. For example, CYP2B6*4 has demonstrated increased activity when exposed to buproprion, but decreased activity when exposed to cyclophosphamide[133], and a substrate specific activity is true for other CYP2B6 alleles as well[134]. It is thinkable that with propfol as a substrate, CYP2B6 and UGT1A9 enzyme activity *in vivo* do not have the same activity as *in vitro*. Further, other substances may affect the activity of the enzymes. Antidepressants are well known inhibitors of CYP2B6[135] and oestrogens have been shown to increase CYP2B6 expression and activity[136,137]. However, in our studies I and II, the patients were on no regular medication, which implies that the risk of unknown substances affecting the propofol metabolizing enzymes was probably relatively small.

In study I, we investigated the metabolite production during the first 20 minutes after propofol bolus to picture the activity of the main propofol degrading enzymes. We did not aim at fully describing propofol metabolism which would have demanded a different study set up with continuous prolonged propofol infusion and a more extensive postoperative blood and urine sampling in order to assess the clearance of the drug. During the first phase after bolus, we detected a peak and thereafter a quickly decreasing propofol concentration (distribution[138]) and rising propofol metabolite levels which did not completely reach a plateau during the time of the study. It can be discussed to what extents the metabolic profile during the first 20 minutes after bolus dose reflects the relationship between metabolism and genotype. The metabolism mainly takes place in the liver and liver metabolism is dependent up on liver blood flow. Liver blood flow in turn is affected by anaesthesia and the circulatory changes occurring during anaesthesia induction. The liver blood flow during propofol anaesthesia has shown both to remain unaltered and increase[30,31,139]. In study I and II, the peroperative hemodynamic parameters were stable and similar between all patients and therefore significant differences in liver blood flow affecting metabolism between individuals are not likely ta have occured. We studied ASA I-II patients only and thus we do not expect

but cannot exclude any impaired liver function, Also, propofol has not been shown to accumulate in patients with liver disease[23].

Propofol is highly protein bound[32], and the role of plasma protein level on free propofol concentration in plasma and clearance of propofol has shown divergent results[140-142]. We did not measure serum albumin is study I or II, but it is not likely that any of the patients had hypoalbuminemia to the point that it would result in an altered pharmacokinetics of propofol.

As a complement to propofol, the patients were given alfentanil. Propfol slows down the clearance of alfentanil and vice versa[143-145]. The reasons for these pharmacokinetic changes are mainly believed to be hemodynamical. All patients in study I and II received a weight-adjusted dose of alfentanil and were hemodynamically stable, and thus it is not likely that differences in propofol pharmacokinetics was caused by any opioid interaction or hemodynamic alterations.

In order to create a more steady state like condition, the patients in study II were receiving a Target Controlled Infusion (TCI) of propofol[146]. The duration of infusion may be considered short, but 20 minutes have shown to be enough to reach pseudo steady state[11,147,148]. Median time of infusion during study II was 21 minutes.

Iohom et al, using similar lengths of propofol infusion, did not find any correlation between induction time, propofol requirement, apparent clearance, and CYP2B6*5, *7 and variants of the GABA-receptor GABRE[11]. Another recent work by Khan et al., was studying the correlation between the need of propofol dose until Loss Of Consciousness (LOC), Return Of Consciousness (ROC), depth of Anaesthesia, propofol concentration and genotypes including variants of CYP2B6, UGT1A9, GABRE. They found no correlation between genotype and variation in propofol pharmacokinetics[148].

GENDER ASPECTS

In the *in vitro* part of study I, a higher CYP2B6 protein expression in the female compared to male HLM was observed, but no correlation between the increased CYP2B6 expression and propofol hydroxylation capacity. In the *in vivo* part, there was no correlation between age and metabolic profile, but as for gender, women had a significantly higher metabolite concentration in plasma for all the UGT1A9 metabolites, but not for the CYP2B6 metabolites or propofol concentration. In study II, with a more even gender distribution than in study I, there were similar results with higher level for all UGT1A9 metabolites and this time also CYP2B6 metabolites in women compared to men.

Has the measured higher metabolite level in women compared to men during the first 20 minutes after propofol bolus and infusion a clinical significance? There are numerous of differences between men and women, which affect the pharmacokinetics and pharmacodynamics of many commonly used drugs[70,73]. Several of these factors could possibly contribute to the gender differences seen with propofol.

Concerning the gender differences in CYP450 pharmacogenetics, an increased CYP3A4 activity is observed in women[66,149]. As for CYP2B6, Lamba et al. found both higher CYP2B6 expression and activity in women HLM[67] but this gender difference could not be shown *in*

vivo[68,150]. Female sex hormone oestrogene have shown to increase the CYP2B6 activity, however it is not known if this increase would affect the metabolism of propofol[137].

The fact that women wake up faster than men despite the same anaesthetic regime[79,80], and that a faster decrease in plasma propofol concentration has been observed in women[77] could be explained by a larger volume of distribution due to a higher percentage of body fat. A similar plasma concentration of propofol resulting in deeper level of anaesthesia observed in men compared to women according to narcotrend, could be due to difference in sensitivity to propofol, in that case an increased propofol sensitivity in men[151] which is observed in several studies[78,152]. This finding however is somewhat contradicted by the fact that the female sex hormone progesterone with GABA-like properties decreases the need for propofol[153]. The quicker emergence and clearance seen in women *could* also be explained by a quicker metabolism of propofol. This however is opposed by the results of Favetta et al. who found no gender difference in propofol metabolism 24 hours after infusion[10]. Although not our study set-up is not thorough enough to demonstrate increased propofol metabolism in women, the finding is new and in line with previous knowledge of gender differences in propofol pharmacokinetics and pharmacodynamics.

PROPOFOL AND POSTOPERATIVE COGNITIVE RECOVERY

Considering assessment method and study groups

Assessment of cognitive reocvery is complex. When choosing which assessment methods to use, we had to consider when and what to measure. The feasibility of the assessment scales was also an important factor to consider since a very extensive and comprehensive questionnaire might be "too much" for the patients to complete. The aim of study III was to assess early as well as intermediate cognitive recovery and also late cognitive recovery up to one week after surgery and anaesthesia, with a focus on possible effects on cognitive recovery by assumed pharmacological remains of the main anaesthetic. A tool capable of objectively evaluating cognitive performance was considered to be the most suitable to evaluate possible remaining pharmacological effects on CNS. However, even though subjective and objective assessment methods do not usually correspond[95], we wanted to include a subjective assessment scale since the patient's self concept of cognitive capacity considerably affects functionality in every day life. The well validated and extensively used Quality of Recovery 40 (QoR40)[154,155] as well as its shorter version QoR 15[122], both include a broad patient assessment of general recovery. However, since we were focusing on cognitive recovery, the CFQ, being a pure cognitive assessment tool was chosen[123].
Since the aim of our study was not POCD, we chose not to use the extensive test battery used for the assessment of Postoperative Cognitive Dysfunction in the POCD studies. Instead we used the PQRS cognitive test domain being clinically more feasible. Also, the PQRS would allow us to make individual assessments and not only evaluate group recovery rates.

We used the PQRS cognitive, physiological, nociceptive and emotional domains only (leaving out "activities of daily living" and "overall patient perspective") in order to pinpoint our focus area and also to keep the questionnaire feasible.

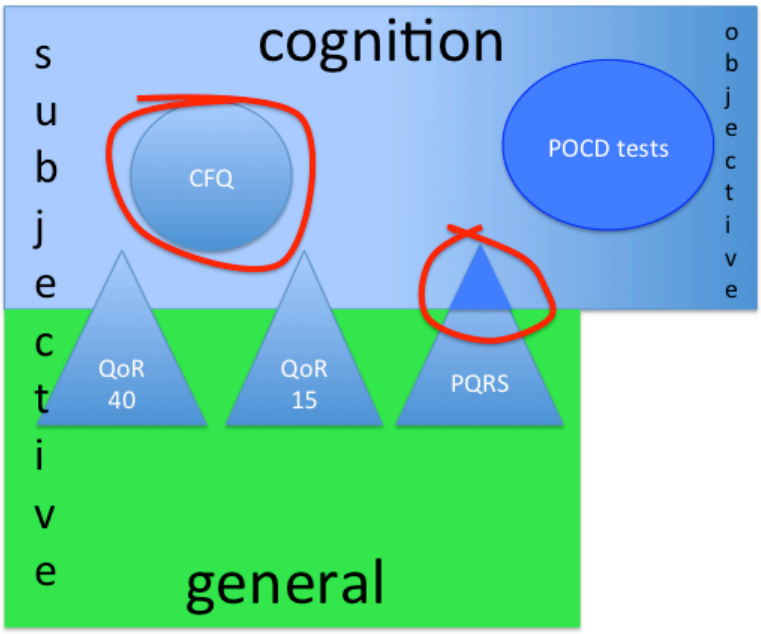


Fig. 4 The figure shows the characteristics of various tools for postoperative assessment of general recovery and cognition. The POCD tools are objectively assessing cognition only, while the quality of recovery tools QoR40 and QoR15 are subjectively assessing general recovery and some cognitive aspects of recovery. The CFQ and a the cognitive (objective), physiological, nociceptive and emotional (subjective) domains of PQRS were used in study III and IV (marked in red).

We chose to study a patient group undergoing ambulatory surgery and therefore in need of a quick cognitive recovery. Longer surgery and anaesthesia would possibly detect more subtile differences in recovery between various anaesthetics, but would also bring more confounding factors such as postoperative pain and higher doses of opioids, which may affect cognitive recovery.

To eliminate the effect on recovery by gender differences, we chose to study women only. Age is a known risk factor of postoperative cognitive decline[4,156], thus we chose the maximum age of the study subjects to be 65 years. A relatively healthy study group was chosen (ASA I-II) to minimize co-morbidity, which could otherwise also possibly affect postoperative recovery[156].

Recovery according to the PQRS

In study III, we found no difference in postoperative cognitive recovery according to PQRS between the propofol or desflurane groups up to 48 hours after surgery and anaesthesia. Nor was there any difference in emergency physiological recovery, nociceptive or emotional recovery between groups. These are results in line with previous observations with no or small differences in cognitive recovery in early recovery[114,116,117]. Thus it seems that after shorter surgery, cognitive recovery after propofol and inhalative anaesthetics is mainly alike. Propofol has been associated with impaired postoperative cognition when compared to inhalative anaesthetics, but the type and size of surgery and anaesthesia in those studies are not comparable to the circumstances of ambulatory surgery and anaesthesia[100,157].

In order to observe a clinically significant delay in cognitive recovery caused by reduced metabolism, the metabolism during redistribution would have to be slow enough to allow the plasma propofol level to reach concentrations high enough to affect the CNS. Using the PQRS recovery assessment tool, we did not observe such as an effect. It can be argued that we should have measured the propofol concentration and metabolite level of the subjects in study III, in order to relate those results to the cognitive recovery. However, regardless of those parameters, any possibly existing difference in propofol concentration or metabolite levels did not affect the cognitive recovery after ambulatory surgery and anaesthesia in a clinically significant way in our material. Whether a different tool would have been able to detect minor cognitive effects cannot be stated. The usefulness of a more detailed and sophisticated method to find very subtile differences in cognitive recovery between groups after ambulatory surgery can be discussed.

Recovery according to the CFQ

In study III, we found no difference in the rate of subjective cognitive recovery between the propofol or desflurane groups according to CFQ. Recovery rate after one week was 65-71 %, suggesting that many of the patients did not experience cognitive recovery at that time. Previous studies using CFQ have found cognitive desorientation in a substantial part of patients as long as 1-2 months after major surgery[158,159], but little or no effect on cognition three days after ambulatory surgery[123 124]. It is expected that recovery after major surgery is prolonged compared to post ambulatory surgery. It is plausible that cognitive recovery is slower after cancer surgery than after non-cancer surgery, due to maintained worry and anxiety.

There was a lack of correlation between subjective and objective recovery in our study, which is in line with other studies[160-163]. A patient grading recovery tool such as the CFQ (or QoR40, QoR15, FRI etc.[122,154,164]) is effective for the assessment of quality of recovery which in turn is of great importance since it may well affect the ability to resume everyday activities as much or more than the objective cognitive recovery[161].

Also, a proper comparison between the PQRS and the CFQ assessment methods was not possible since the tools were used at different time points; 20 and 48 hours for the PQRS and 72 hours and 1 week for the CFQ. The reason for the CFQ being relatively postponed was for the patients to have time to experience the every-day situations asked for in the questionnaire. Also even though we requested the patients to leave out questions including situations they had not yet confronted postoperatively, almost all questionnaires were completed. Thus, it is plausible that some of the situations were not truly experienced but merely answered.

COGNITIVE PERFORMANCE AND PQRS

Aspects on PQRS cognitive baseline performance

We observed that many patients in both the propofol and desflurane group considerably improved their cognitive score at 20 hours postoperatively compared to baseline. The improvement was most pronounced for "word recall". Berman et al. observed impaired verbal working memory in pre-treatment cancer patients, and a correlation between impaired cognitive performance, high level of worry and altered brain function in magnetic resonance imaging (MRI)[165]. We reasoned that the improved postoperative cognition in study III could

be a result of relief and decreased worry after completed surgery, and that the cognitive recovery performance of these patients might be a reflection of "pre-and post surgery worry and anxiety", rather than of possible remaining effects of the anaesthetic agent.
Cognitive impairment in women with breast cancer is commonly observed, and has been often been regarded as a consequence of chemotherapy treatment[166]. However, in several studies, impaired objective cognition has been observed *before* chemo treatment as well, suggesting that factors associated with the malignant diagnosis, such as increased anxiety, depression and sleeplessness contribute to the cognitive impairment [165,167-169].

Since the cognitive recovery according to PQRS is dependent on the return to baseline result, a reliable baseline score is of great importance when using this scale. A cognitive baseline test is recommended to take place at least two weeks before surgery in order to minimize influence by preoperative anxiety[170], which is commonly seen in patients waiting for both major and minor surgery[171-173]. Due to logistic reasons, we sometimes performed our baseline testing as close as one week before surgery, which may have affected the baseline results negatively. Our study groups had both a malignant disease and were waiting for surgery, two factors known to cause anxiety, worry and with that possibly affected cognition.

"Normal variation" in cognitive performance

Royse et al. changed the scoring system of PQRS to allow for the day-to-day normal variation in cognitive performance found in healthy individuals. With the new score allowance, 80 % of the volunteers were "recovered" in a test re-test situation. Therefore a group performing the cognitive domain of PQRS and reaching a recovery rate of 80% correspond to the normal variation in cognitive performance seen in healthy volunteers[127]. We hypothesised that anxiety and worry in patients waiting for cancer surgery would affect cognitive performance and the "normal variation" in cognitive performance compared to healthy volunteers. Lack of observation and awareness of variation in cognitive performance is highlighted as a problem in studies on POCD, and thus a source of erroneous conclusions[94,174]. The same erroneous conclusions may be committed in studies on cognitive recovery if ignoring the existence of normal variation in cognitive performance between subsequent test sessions. To better understand if we had studied cognitive performance and recovery influenced by anxiety and worry more than influenced by choice of anaesthetics, we performed study IV.

As the revised PQRS scoring system recommends that patients with a baseline score equal to or lower than the allowed tolerance factor for each question is excluded (since they will otherwise be recovered at re-test no matter how bad the performance), the number of patients compared to controls that would fail to perform a baseline score good enough to be included in the test was studied. The exclusion number along with the variation in cognitive performance measured as change in score between re-tests over time would give an indication of how cognitively affected the patient group would be compared to healthy subjects. Moreover, the proportion of patients compared to controls succeeding in fulfilling the criteria for "recovery" would indicate a possible difference in variability in cognitive performance between the groups. Nociception and emotional domains of PQRS were studied in parallell.

We found that a significantly larger proportion of the patients were excluded due to low baseline performance and that the patients expressed higher level of anxiety than controls.

The change in score at re-tests was similar between the groups. Exclusion rates of 11-28% due to poor baseline test performance are also seen in other patient studies using the cognitive domain of PQRS. Interestingly, this large preoperative exclusion rate has not been commented on in any of the studies[86,87,129]. In the desflurane and propofol groups in study III, none of the patients were excluded due to low baseline score since this regime was not published at the writing of the article. If recalculating the baseline data, five patients in the desflurane and four patients in the propofol group would have been excluded due to low baseline score. This is a slightly lower proportion than in the patient group but a larger proportion than in the control group in study IV. Fig. 5 below shows the baseline score data of the subjects in study III and IV, and the number of patients excluded in each question according to the revised PQRS. Some patients are excluded in several of the questions.

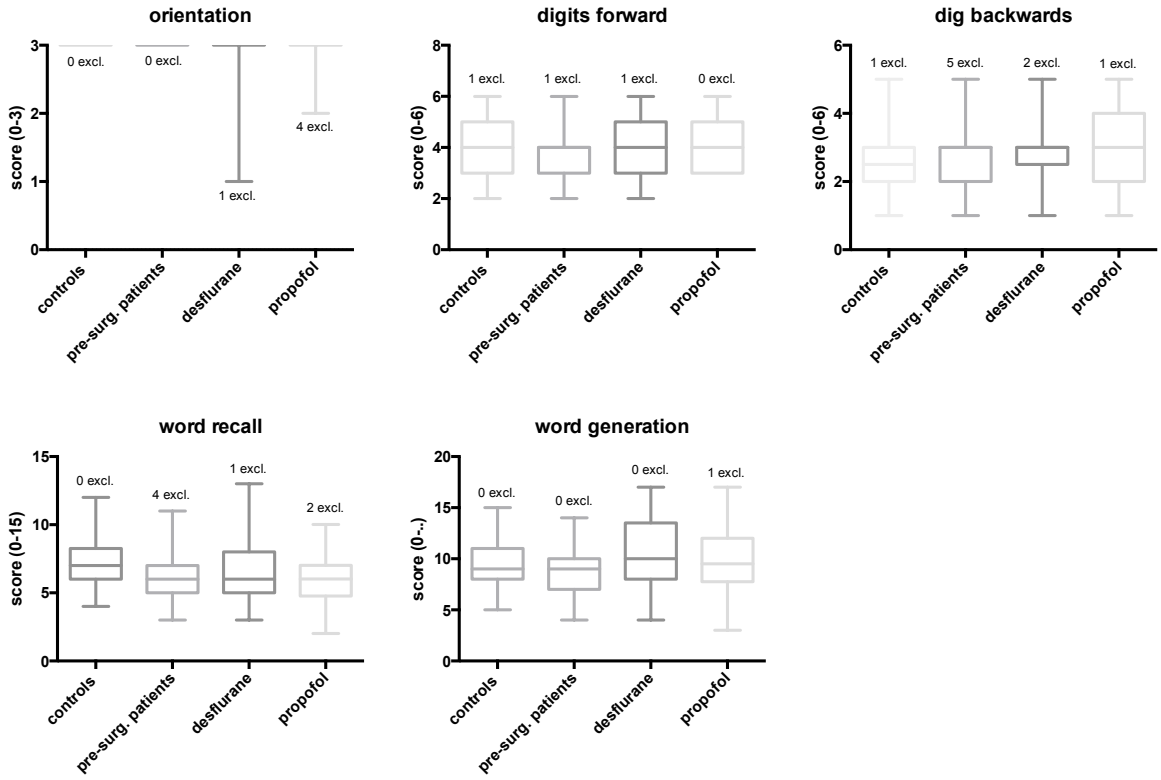


Fig. 5 The figure shows the median, interquartile range, max and min absolute score for all cognitive questions for the patients and controls in in study III (desflurane, propofol) and IV (controls, pre-surgery patients). The number of subjects excluded due to low baseline score in each group for each question is presented next to each respective box. The score on the Y-axis is the maximum score obtainable for that question. There is no maximum score for "word generation". There is no significant difference in median absolute score between the groups.

Even though the exclusion rate in the desflurane and propofol groups was not significantly higher than in the control group, it is striking that five patients in those groups failed to answer the "orientation" questions; "what is your name, in which town are you now, when were you born" (all patients in study IV scored a maximum in this question). Failing this question is most likely a result of stress.

Emotional distress before and after cancer surgery

We compared the nociceptive and emotional absolute scores of all the patient groups in study III and IV ("pre-surg. patients", desflurane and propofol groups) to the absolute score of the control group at all time points (Fig. 6).

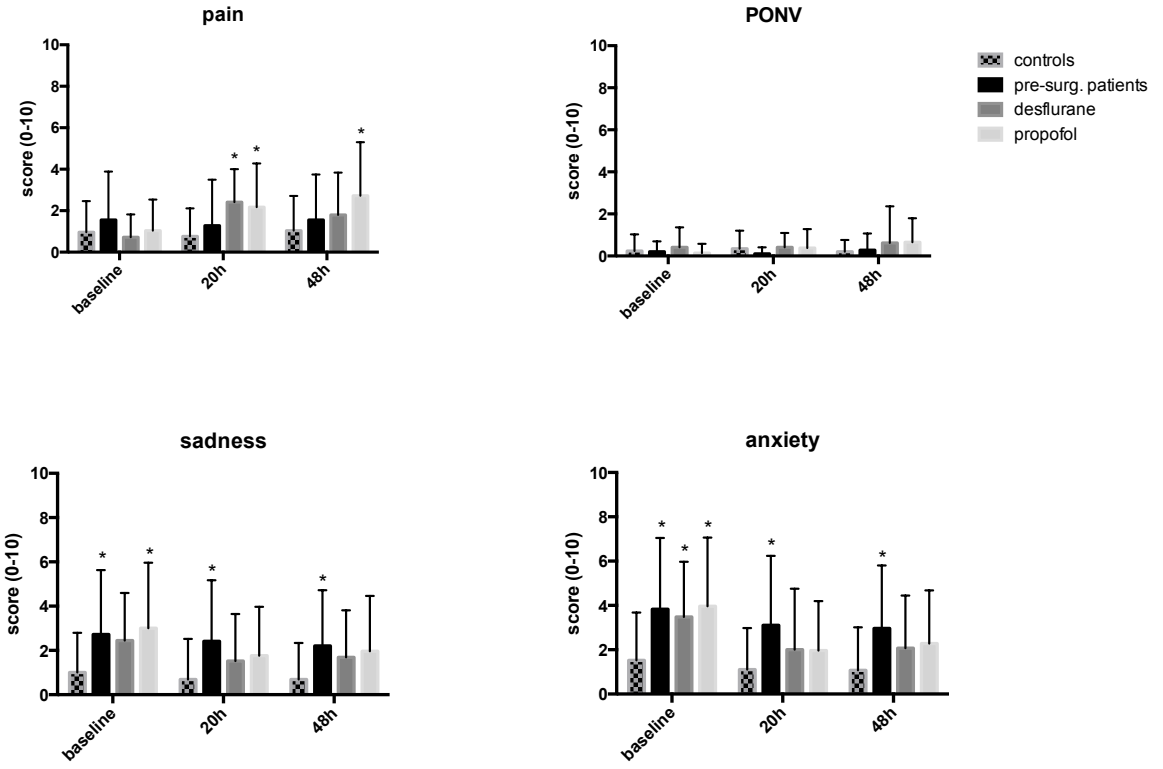


Fig. 6 The figure above shows the nociceptive (pain, PONV) and emotional (sadness, anxiety) domains of the PQRS for all the studied groups in study III and IV.p* <0.05 is considered significant. RM ANOVA is used to compare patient groups to control group score. The time on the X-axis is from end of surgery/anaesthesia (desflurane, propofol, paper III) and from baseline (pre-surg. patients, controls, paper IV) PONV; PostOperative Nausea and Vomiting.

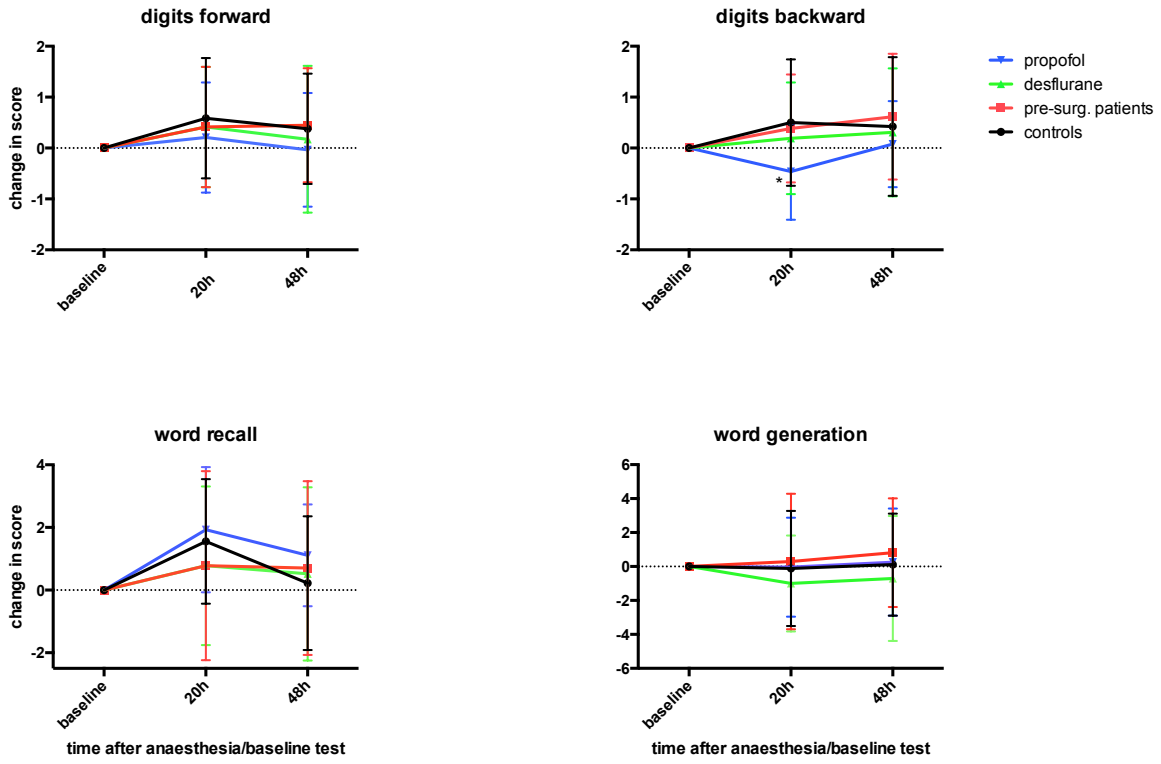
We found the emotional distress to be generally higher in the patient groups at baseline, but that once surgery was performed, only the pre-surgery patients maintained a higher sadness and anxiety-level compared to controls. This is an interesting finding; the level of emotional distress seems to decrease as a result of completed surgery but is maintained in the non-operated group of patients. This may be one of the reasons for some patients' markedly improved cognitive performance after surgery in study III. It is conceivable that the high sadness and anxiety level contributed to the low baseline score in the pre-surgery patient group. It is also thinkable that the large exclusion rate from the cognitive assessment due to low baseline cognitive test score observed in the other studies using the PQRS, could be partly caused by a high level of preoperative stress and worry. It is not known how large a proportion of the patients in other PQRS studies were waiting for cancer surgery, which might increase the preoperative anxiety. It is noticeable that the rate of emotional recovery tells relatively little about the actual level of anxiety and sadness, since that level may be high both pre-and postoperatively and in that case results in a "good recovery" even though the patient is emotionally very distressed.

The PQRS nociceptive domain

"Pain" was as expected significantly higher in the operated groups when compared to controls. There was no difference between the groups with regard to PONV, which suggests that anti-emetic prophylaxis given according to risk factors results in no increased PONV risk after desflurane anaesthesia compared to propofol anaesthesia after shorter breast surgery, even though volatile anaesthesia is more prone to provoke nausea and vomiting[6,17,72].

Change in PQRS cognitive score at test re-test

There was no difference in change in score at test re-test between control and pre-surgery patients, and when we compared all groups from study III and IV, the similarity between groups remained except for "digits backward" at 20 hours (propofol vs. controls/pre-surgery patients).

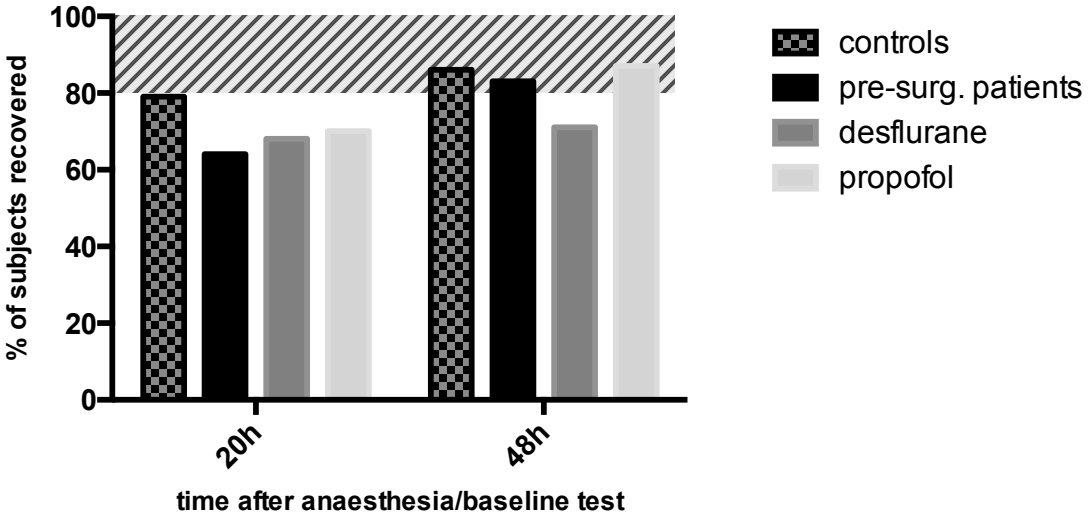


The figure shows the combined results of mean change in score and standard deviation from baseline to respective measurement point for each cognitive question (not orientation due to maximum score for both groups in study IV) of the PQRS from paper III (desflurane and propofol) and paper IV (pre-surg. patients and controls) at 20 h and 48 h after anaesthesia/surgery (paper III) or baseline test (paper IV). *p< 0.05 at "digits backward" 20h for controls vs propofol and pre-surg. patients vs. propofol. PQRS; Postoperative Quality of Recovery Scale.

A small positive change in score as a result of a learning effect is an expected result over time in cognitively unaffected subjects in a test re-test situation, with the largest change from the first to the second test[127]. A lack of improvement in score over time is suggested to be interpreted as a sign of cognitive impairment[94]. In both study III and IV, the overall trend is a higher score at 48 hours than at baseline. The propofol group had a negative change in score at 20 h for "digits backward", but also the largest positive change in score for "word recall", and cannot be considered more cognitively affected than any other group. There is a trend of the controls having a smaller SD than the patient groups in all questions, which may be interpreted as a more consistent cognitive test performance in that group. The difference is merely a trend.

Fulfilling the PQRS "recovery" criteria

To study to what extent malignancy, surgery and anaesthesia seemed to affect the ability to fulfil the cognitive "recovery" criteria of the PQRS, we compared the proportion reaching "recovery" between all the patient groups and the controls.



The figure shows the combined results of cognitive recovery according to PQRS from paper III (desflurane and propofoI) and paper IV (pre-surg. patients and controls) at 20 h and 48 h after anaesthesia/surgery (paper III) or baseline test (paper IV). The stripe-shaded field represents the area of "good recovery" according to Royse et al. (Anesthesiology 2013). PQRS; Postoperative Quality of Recovery Scale.

Interestingly, at 20 hours, the "recovery" rate in the pre-surgery group was similar to the newly operated groups, only the healthy control group reached "good recovery" rate of 80%. All groups reached "good recovery" at 48 hours except for the desflurane group. The differences in "recovery" between groups are relatively small, and should be interpreted carefully since several factors beyond our control and knowledge are likely to affect recovery[127]. However, it seems that preoperative stress can affect the abililty to fulfil the PQRS "recovery" criteria as much as ambulatory surgery and anaesthesia.

In general, when studying postoperative cognitive recovery in patients who are likely to be influenced by several factors affecting cognition such as severe disease, all factors have to be taken into consideration when assessing baseline performance and choosing assessment tool. If the study intends to, as in our case, study the effect on cognition of remaining anaesthetics, the impact of other factors can overwhelm and even eliminate the effect of what is really intended to be studied. If the positive effect on cognitive performance by for example relieve that surgery is over, is greater than the possible negative effects of residual anaesthetic effects, then the net effect will be a relative improved cognitive performance.

CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

The postoperative recovery depends on various factors, and those factors vary in between patients. The aim for the anaesthetist is to tailor the anaesthesia to each patient. Even though most patients receive properly adjusted propofol anaesthesia, some patients may risk being underdosaged or receiving an overdose. Knowing when and how to adjust our anaesthesia would simplify the adjustment to the individual patient.

In this thesis, we found no correlation between studied genotypes and propofol metabolite concentration in plasma. Since the genotypes seemingly involved in increased and decreased propofol metabolism are rare, a considerably larger study including thousands of patients would be needed in order to enable any conclusions on correlation between genotype and propofol metabolism. The consequences of anaesthetising a fast or a slow metaboliser without knowing it may have serious consequences for the individuals affected, even though they are few. Awareness is a risk from undertreatment while prolonged recovery, depressed breathing and hemodynamic instability are risks of an overdose. The right way to achieve the knowledge of how to individualise propofol anaesthesia may not be through clinical patient studies since the size of the studies have to be very large in order to find the possible few slow and fast individuals. A genetic mapping of individuals is a more realistic way to search for the probable outliers. It would also be possible to study the genotype and propofol metabolism in patient outliers that either demand very large doses of propofol, or have a significantly prolonged recovery after propofol anaesthesia.

The finding that women had a higher propofol metabolite production in plasma compared to men is in line with the many clinical observations of gender difference in response to propofol. Women wake up faster than men after propofol anaesthesia, women demand a higher propofol dose than men to maintain the same level of anaesthetic depth, and women experience awareness more often than men. The reasons for these findings may be pharmacokinetical and/or pharmacodynamical. Even though our finding is not conclusive, it points out the existing gender differences in response to anaesthetic drugs, of which many are not fully explained[2,70]. A larger study with more extended surgery could further clarify if there is really a gender difference in propofol metabolism.

It has to be carefully considered which recovery assessment tool to use when evaluating postoperative cognitive recovery. In our studies, the modified version of PQRS has been shown to detect varying levels of worry and anxiousness in a patient population, which may be of importance for the general recovery (and cognitive recovery) of the individual. The PQRS is one of few tools including objective cognitive tests, which is a great advantage of the scale. However, the scale commonly seems to exclude a considerable part of patients from cognitive assessment due to low baseline score, and that has to be considered. It may be that that the excluded part of patients is the proportion with the highest risk of slow cognitive recovery, and in that case in need of particular observation.

Possible differences in propofol pharmacokinetics and dynamics did not seem to affect the cognitive recovery after ambulatory surgery and anaesthesia. Increasing the time and size of surgery would increase the risk of confounding factors and the conclusion would be difficult to interpret.

Maybe future studies should focus more on possible association between anaesthestics, postoperative cognitive recovery and neurocognitive side effects including POCD. Hovens et al. emphasise the need to fill the gap between preclinical and clinical perspectives of POCD[175]. The transition between a ”normal cognitive recovery” and a pathological cognitive state is delicate and when it occurs is unknown. The “Pinocchio trial” is an ongoing investigation of the effect of various anaesthetic regimes including propofol, on the development of postoperative delirium (PD), early postoperative cognitive dysfunction (up to 60 minutes postoperatively) and if delayed postoperative recovery increases risk of delirium[176]. The interim results show that the risk for PD is higher after propofol anaesthesia compared to sevoflurane and desflurane (Doronzio A, Eur J of Anaesthesiology, 2013). This calls for further studies on propofol and cognitive recovery.

CONCLUSIONS

With this thesis we have investigated genetic and cognitive aspects on variation in response to the intravenous anaesthetic propofol. The genetic studies were performed on human liver tissue as well as on patient material, and the studies on cognitive recovery were conducted in a clinical setting and in comparison with desflurane. The conclusion in detail:

- None of the studied genotypes of CYP2B6 *4,*5 or *6 showed any correlation to propofol hydroxylation capacity in human liver microsomes. Female livers expressed an increased amount of CYP2B6, but this was not correlated to increased hydroxylation capacity of propofol.

- There was a considerable interindividual variation of propofol metabolite level in plasma but no correlation between metabolite level and any of the studied genotypes of CYP2B6 and UGT1A9 after bolus dose or short infusion. Females had a higher level of UGT1A9 generated metabolites after propofol bolus dose, and of both UGT1A9 and CYP2B6 generated metabolites after short propofol infusion. Age had no impact on metabolite level.

- There was no difference in cognitive recovery up to one week postoperatively after propofol or desflurane anaesthesia in female patients undergoing ambulatory surgery.

- Women with malignant disease and waiting for surgery performing the cognitive, nociceptive and emotional test re-test of the PQRS expressed a higher level of anxiety, had a worse cognitive performance at baseline but showed no difference in cognitive change in score at re-tests when compared to healthy controls.

"Everything is going to be fine in the end. If it is not fine, it is not the end."

Unknown

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Det är många individer som på olika sätt har varit inblandade i denna mångfacetterade och oerhört lärorika resa;

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